



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/705, C12N 15/81, 1/19, C12Q 1/02	A1	(11) International Publication Number: WO 96/13520 (43) International Publication Date: 9 May 1996 (09.05.96)
(21) International Application Number: PCT/US95/14364 (22) International Filing Date: 25 October 1995 (25.10.95) (30) Priority Data: 08/332,312 31 October 1994 (31.10.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/332,312 (CIP) Filed on 31 October 1994 (31.10.94) (71) Applicant (for all designated States except US): AMERICAN CYANAMID COMPANY [US/US]; Five Giralda Farms, Madison, NJ 07940 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): PAUSCH, Mark, Henry [US/US]; 312 Andover Place, Robbinsville, NJ 08692 (US). PRICE, Laura, Alicia [US/US]; 181 Canterbury Avenue, Langhorne, PA 19047 (US). (74) Agents: MATHEWS, Gale, F.; American Home Products Corporation, Five Giralda Farms, Madison, NJ 07940 (US) et al.		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: GENES ENCODING A FAMILY OF POTASSIUM CHANNELS (57) Abstract This invention relates generally to the potassium channel gene family. More particularly, the present invention relates to the cloning and characterization of potassium channel genes from <i>Drosophila melanogaster</i> and <i>Caenorhabditis elegans</i> . Other aspects of the present invention include methods of assaying substances to determine effects on cell growth. Also presented are methods of controlling nematode and insect pests by inhibiting potassium channels substantially homologous to those encoded by nucleotide sequences as described herein.		

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GENES ENCODING A FAMILY OF POTASSIUM CHANNELS

Field of Invention

This invention relates generally to the potassium channel gene family. More particularly, the present invention relates to the cloning and characterization of potassium channel genes from *Drosophila melanogaster* and *Caenorhabditis elegans*.

Background of the Invention

Synthetic organic insecticides are primarily nerve poisons acting on the cholinergic system (organophosphorus compounds and methylcarbamates), the voltage-gated sodium channel (pyrethroids and DDT), and the GABA-gated chloride channel (cyclodienes and other polychlorocycloalkanes). Potassium channels comprise a large and diverse group of integral membrane proteins that determine the level of excitability and repolarization properties of neurons and muscle fibers [B. Hille, *Ionic Channels of Excitable Membranes*, Sinauer, Sunderland, MA (1984)]. The multiple essential functions encoded by the potassium channels make them excellent targets for new pesticides and animal and human therapeutics. Potassium channel diversity in the fruitfly *Drosophila melanogaster* results from an extended gene family coding for homologous proteins. Six genes encoding

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potassium channels have been cloned from *Drosophila melanogaster* which account for a large part of the diversity of potassium currents observed in insect nervous tissue [A. Wei, M. Covarrubias, A. Butler, K. Baker, M. Pak, L. Salkoff, *Science* 248, 599-603 (1990), N.S. Atkinson, G.A. Robertson, B. Ganetzky, *Science* 253, 551-555, (1991), J. Warmke, R. Drysdale, B. Ganetzky, *Science* 252, 1560-1564 (1991), A. Bruggemann, L.A. Pardo, W. Stuhmer, O. Pongs, *Nature* 365, 445-448 (1993)]. *Shaker* and *Shal* encode voltage-gated potassium channels with rapid current activation and inactivating properties. *Shab* and *Shaw* encode delayed rectifier channels, with slow inactivating (*Shab*) and non-inactivating (*Shaw*) properties. *Slo* encodes a calcium-activated potassium channel and *eag* encodes a voltage-gated channel permeable to both potassium and calcium which is modulated by cyclic AMP.

Modulation of cardiac action potential by compounds that effect the behavior of potassium channels may be a useful treatment for serious heart conditions. In this regard, each of the potassium channels cloned from insects have corresponding versions in mammalian species, including, specifically, a delayed rectifier potassium channel homolog, RAK, cloned from rat cardiac tissue [M. Paulmichl, P. Nasmith, R. Hellmiss, K. Reed, W.A. Boyle, J.M. Nerbonne, E.G. Peralta, D.E. Clapham, *Proc. Natl. Acad. Sci USA* 88, 7892-7895 (1991)]. Thus, the RAK channel represents an important target of new drugs for the control of heart failure. The delayed rectifier potassium current in heart cells regulates the duration of the plateau of the cardiac action potential by countering the depolarizing,

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inward calcium current. Delayed rectifier potassium currents characteristically are activated upon depolarization from rest, display a sigmoidal or delayed onset, and have a nonlinear, or rectifying, current-voltage relation. Several types of delayed potassium conductances have been identified in cardiac cells based on measured single-channel conductances. Heart rate and contractility are regulated by second messenger modification of delayed rectifier potassium conductances, and species differences in the shape of the plateau may be influenced by the type and level of channel expression.

On the basis of predicted membrane spanning topology, potassium channels may be subdivided into two distinct classes: voltage-gated, calcium-activated, and cyclic nucleotide-gated potassium channels that are composed of six membrane spanning domains (S1-S6) and a single pore forming domain (H5), and inward rectifying potassium channels that pass through the membrane twice and also contain a single pore forming region [Y. Kubo, E. Reuveny, P.A. Slesinger, Y.N. Jan, L.Y. Jan *Nature* 364, 802-806 (1993); Y. Kubo, T.J. Baldwin, Y.N. Jan, L.Y. Jan *Nature* 362, 127-133 (1993)]. Here, we report the cloning and functional expression in yeast of a novel *Drosophila melanogaster* potassium channel. Further, we identify a *Caenorhabditis elegans* homolog that constitutes the second member of a new family of potassium channels exhibiting a topological configuration unique among the known classes of potassium channels.

The yeast *Saccharomyces cerevisiae* is utilized as a model eukaryotic organism for the purpose of studying potassium transport mechanisms.

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Due to the ease with which one can manipulate the genetic constitution of the yeast *Saccharomyces cerevisiae*, researchers have developed a detailed understanding of many complex biological pathways, including potassium transport. In yeast, high affinity potassium uptake is performed by the product of the *TRK1* gene [R.F. Gaber, C.A. Styles, G.R. Fink *Mol. Cell. Biol.* 8, 2848-2859 (1988)]. Mutant yeast strains lacking *trk1* function are incapable of growing in medium lacking high concentrations of potassium. Since potassium transport mechanisms are present in organisms as divergent as yeast and man, one could predict that expression of heterologous potassium channels in mutant cells might replace *trk1* function, and support growth on medium containing low potassium concentration. In this regard, plant potassium channels were shown to function in yeast and represent important targets for new herbicides [J.A Anderson, S.S. Huprikar, L.V. Kochian, W.J. Lucas, R.F. Gaber, *Proc. Natl. Acad. Sci USA* 89, 3736-3740 (1992); H. Sentenac, N. Bonnaud, M. Minet, F. Lacroute, J.-M. Salmon, F. Gaynard, C. Grignon, *Science* 256, 663-665 (1992); D.P. Schachtman and J.I. Schroeder, *Nature* 370, 655-658]. Thus, we have employed this yeast expression system for cloning and expression of potassium channels from heterologous species, making it useful for discovery of new pesticides, and animal and human therapeutics. Discovery of such compounds will necessarily require screening assays of high specificity and throughput. For example, new pesticides directed at potassium channels require high selectivity for insect channels and low activity against non-insect species. Screening assays utilizing yeast strains genetically modified to

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accommodate functional expression of heterologous potassium channels offer significant advantages in this area.

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Summary of the Invention

A first aspect of the present invention is the discovery of a new subclass of potassium channel genes and proteins encoded thereby. Potassium
10 channels belonging to this new subclass comprise four hydrophobic domains capable of forming transmembrane helices, wherein a first pore-forming domain is interposed between the first and second transmembrane
helices and a second pore-forming domain is interposed
15 between the third and fourth transmembrane helices, and wherein each pore-forming domain contains a potassium selective peptide motif. In preferred embodiments, the peptide motif is selected from the group consisting of a Y/F-G dipeptide motif.

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In certain preferred embodiments, the isolation and characterization of invertebrate (i.e. insect and nematode) potassium channel genes belonging to this new subclass is presented. In more preferred
embodiments, the present invention provides for the
25 isolation of complementary DNA fragments from *Drosophila melanogaster* and *Caenorhabditis elegans* which encode conserved amino acid sequence elements unique to this potassium channel gene family. A yeast expression technology is employed to clone cDNAs from
30 *Drosophila melanogaster* and *C. elegans* and a hybridization approach is utilized to isolate additional cDNAs from *Caenorhabditis elegans*.

A second aspect of the present invention is a method of assaying substances to determine effects

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on cell growth. Yeast cells of the kind described above are cultured in appropriate growth medium to cause expression of heterologous proteins, embedded in agar growth medium, and exposed to chemical compounds applied to the surface of the agar plates. Effects on the growth of embedded cells are found around compounds that have effects on the heterologous potassium channel.

A third aspect of the present invention is a method of controlling nematode and insect pests by inhibiting potassium channels substantially homologous to those encoded by nucleotide sequences as presented herein.

Brief Description of the Drawings

FIGURE 1. Growth of CY162 cells bearing pDmORF1. CY162 cells transformed with plasmids isolated from survivors of a primary library screen for plasmids that support the growth of CY162 on medium contain low potassium concentration. Six individual transformants of each plasmid-bearing strain are cultured in patches on the indicated medium. CY162 cells bearing pDmORF1 are found in the upper left-hand corner of each plate while pKAT1 containing cells are found in the lower right hand corner.

FIGURE 2A and 2B. DNA sequence and deduced amino acid sequence of Dm ORF1 [SEQ ID NOS:1 and 2]. The nucleotide sequence of the 2.4 kb cDNA revealed a single long open reading frame proximal to the GAL1 promoter. Segments corresponding to putative transmembrane (M1-M4) and pore-forming H5 domains in the predicted polypeptide are underlined. The single

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amino-terminal asparagine linked glycosylation site is indicated by a G.

FIGURE 3A and 3B. DNA sequence and deduced amino acid sequence of the F22b7.7 segment of the *Caenorhabditis elegans* genome [SEQ ID NO:3]. Segments corresponding to putative transmembrane (M1-M4) and pore-forming H5 domains in the predicted polypeptide are underlined.

FIGURE 4. Alignment of DmORF1 and F22b7.7 sequences. Protein-coding regions of DmORF1 [SEQ ID NO: 37] and F22b7.7 [SEQ ID NO: 38] (designated as CeORF-1 in this FIGURE) are compared using the protein sequence alignment algorithm in Genework DNA sequence analysis software. Identical amino acids are boxed.

FIGURE 5A. Comparison of the pore-forming domains of DmORF1 and F22b7.7. Amino acid sequences from the six cloned *Drosophila melanogaster* potassium channels and three inward rectifier channels [SEQ ID NOS:7 through 21] are compared to DmORF1 and F22b7.7 within the pore-forming H5 regions. Amino acid identities are indicated by a vertical line and conserved substitutions indicated by a dot. Amino acid substitutions deemed acceptable are indicated.

FIGURE 5B. Hydropathy plot analysis of the DmORF1 and F22b7.7 polypeptide sequence. The Kyte-Doolittle hydropathy algorithm in the Geneworks DNA analysis software is used to predict the topology of DmORF1 and F22b7.7. The position of predicted membrane spanning domains (M1-M4) and pore-forming domains are indicated.

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FIGURE 6. Predicted membrane spanning topology of DmORF1.

FIGURE 7. Heterologous potassium channel-dependent growth of plasmid bearing CY162 (*trk1Δ*) strains. CY162 bearing pYES2, pKAT1, pDmORF1, and pRATRAK are cultured at 30°C for four days on arginine phosphate agar medium containing 0 mM, 0.2 mM, or 100 mM added KCl.

FIGURE 8. Inhibition of growth of yeast cells containing heterologous potassium channels. CY162 cells (10^5) bearing the indicated plasmids are plated in arginine phosphate agar medium containing 0.2 mM potassium chloride. Sterile filter disks were placed on the surface of the agar and saturated with 20 ml of a 1 M solution of potassium channel blocking compound. Clockwise from upper left-hand corner is BaCl_2 , CsCl , TEA, and RbCl . KCl is applied to the center disk.

FIGURE 9A and 9B. DNA sequence and deduced amino acid sequence of CORK [SEQ ID NO: 36]. The nucleotide sequence of the 1.4 kb cDNA revealed a single long open reading frame proximal to the *GAL1* promoter. Segments corresponding to pore-forming H5 domains in the predicted polypeptide are underlined. Asparagine-linked glycosylation sites are indicated by a G.

Detailed Description of the Invention

Nucleotide bases are abbreviated herein as follows:

Ade; A-Adenine G-Guanine Ura; U-Uracil

C-Cytosine T-Thymine

Amino acid residues are abbreviated herein to either three

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letters or a single letter as follows:

Ala;A-Alanine Leu;L-Leucine

Arg;R-Arginine Lys;K-Lysine

Asn;N-Asparagine Met;M-Methionine

5 Asp;D-Aspartic acid Phe;F-Phenylalanine

Cys;C-Cysteine Pro;P-Proline

Gln;Q-Glutamine Ser;S-Serine

Glu;E-Glutamic acid Thr;T-Threonine

Gly;G-Glycine Trp;W-Tryptophan

10 His;H-Histidine Tyr;Y-Tyrosine

Ile;I-Isoleucine Val;V-Valine

The term "mammalian" as used herein refers to any mammalian species (e.g., human, mouse, rat, and monkey).

15 The term "heterologous" as used herein refers to DNA sequences, proteins, and other materials originating from organisms other than the organism used in the expression of the potassium channels or portions thereof, or described herein (e.g., mammalian, avian, amphibian, insect, plant),
20 or combinations thereof not naturally found in yeast.

The terms "upstream" and "downstream" are used herein to refer to the direction of transcription and translation, with a sequence being transcribed or translated prior to another sequence being referred to as "upstream" of the
25 latter.

The potassium channels of the present invention possess properties in common with known potassium channels including, voltage-gated channels, calcium activated channels, cyclic nucleotide gated channels, inward rectifier
30 channels, and the like, and especially with regard to electrophysiological properties. Certain preferred channels exhibit inward and outward currents that are affected by potassium concentration, particularly characteristic of voltage-gated channels. The term "channel" and the

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nucleotide sequences encoding same, is intended to encompass subtypes of the aforementioned classes of channels, and mutants, derivatives and homologs thereof.

5 The nucleotide sequences encoding the potassium channels or parts thereof may be expressed recombinantly, and utilized for a variety of reasons, the most notable of which is for screening of substances that modulate the activity of the potassium ion channels. Such substances, especially inhibitors of the activity of the potassium
10 channels of the present invention, may be utilized as insecticides, antihelmenthics, drugs suitable for the control of heart failure, and the like.

Heterologous DNA sequences are typically expressed in a host by means of an expression vector. An expression vector
15 is a replicable DNA construct in which a DNA sequence encoding the heterologous DNA sequence is operably linked to suitable control sequences capable of affecting the expression of a protein or protein subunit coded for by the heterologous DNA sequence in the intended host. Generally,
20 control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and (optionally) sequences which control the termination of transcription and translation. Vectors useful for
25 practicing the present invention include plasmids, viruses (including bacteriophage), and integratable DNA fragments (i.e., fragments integratable into the host genome by genetic recombination). The vector may replicate and function independently of the host genome, as in the case of
30 a plasmid, or may integrate into the genome itself, as in the case of an integratable DNA fragment. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. For example, a promoter operable in a host cell is

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one which binds the RNA polymerase of that cell, and a ribosomal binding site operable in a host cell is one which binds the endogenous ribosomes of that cell.

DNA regions are operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leader sequences, contiguous and in reading phase.

Transformed host cells of the present invention are ~~cells which have been transformed or transfected with the~~ vectors constructed using recombinant DNA techniques and express the protein or protein subunit coded for by the heterologous DNA sequences. In preferred embodiments, the transformed host cells are yeast. A variety of yeast cultures, and suitable expression vectors for transforming yeast cells, are known. See e.g., U.S. Patent No. 4,745,057; U.S. Patent No. 4,797,359; U.S. Patent No. 4,615,974; U.S. Patent No. 4,880,734; U.S. Patent No. 4,711,844; and U.S. Patent No. 4,865,989. *Saccharomyces cerevisiae* is the most commonly used among the yeasts, although a number of other yeast species are commonly available. See e.g., U.S. Patent No. 4,806,472 (*Kluveromyces lactis* and expression vectors therefore); 4,855,231 (*Pichia pastoris* and expression vectors therefore). A heterologous potassium channel may permit a yeast strain unable to grow in medium containing low potassium concentration to survive [CY162, for example, see J.A Anderson, S.S. Huprikar, L.V. Kochian, W.J. Lucas, R.F. Gaber, *Proc. Natl. Acad. Sci USA* 89, 3736-3740 (1992)]. Yeast vectors may contain an origin of replication from the endogenous 2 micron (2 μ) yeast plasmid or an autonomously

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replicating sequence (ARS) which confer on the plasmid the ability to replicate at high copy number in the yeast cell, centromeric (CEN) sequences which limit the ability of the plasmid to replicate at only low copy number in the yeast cell, a promoter, DNA encoding the heterologous DNA sequences, sequences for poly-adenylation and transcription termination, and a selectable marker gene. An exemplary plasmid is YRp7, (Stinchcomb et al., (1979) *Nature* 282, 39; Kingsman et al., (1979) *Gene* 7, 141; Tschemper et al., (1980) *Gene* 10, 157]. This plasmid contains the TRP1 gene, which provides a selectable marker for a mutant strain of yeast lacking the ability to grow in the absence tryptophan, for example ATCC No. 44076. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for metallothionein (YEpl52), 3-phosphoglycerate kinase [pPGKH, Hitzeman et al., (1980) *J. Biol. Chem.* 255, 2073] or other glycolytic enzymes [pYSK153, Hess et al., (1968) *J. Adv. Enzyme Reg.* 7, 149]; and Holland et al., (1978) *Biochemistry* 17, 4900], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-fructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPO Publn. No. 73,657. Other promoters, which have the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2 (pAD4M), isocytochrome C, acid phosphates, degradative enzymes associated with nitrogen metabolism, and the aforementioned metallothionein and glyceraldehyde-3-

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phosphate dehydrogenase, as well as enzymes responsible for maltose and galactose (pYES2) utilization. Finally, in constructing suitable expression plasmids, the termination sequences associated with these genes may also be ligated into the expression vector 3' of the heterologous coding sequences to provide polyadenylation and termination of the mRNA.

In one embodiment of the present invention, a yeast expression system is described, wherein yeast cells bear heterologous potassium channels. In preferred embodiments, these channels are DmORF-1, CORK, or RAK. As noted above, transformed host cells of the present invention express the proteins or proteins subunit coded for by the heterologous DNA sequences. When expressed, the potassium channel is located in the host cell membrane (i.e., physically positioned therein in proper orientation for both the stereoselective binding of ligands and passage of potassium ions).

In certain preferred screening embodiments of the present invention, a transformed yeast cell is presented, containing a heterologous DNA sequence which codes for a rat cardiac delayed rectifier potassium channel, RAK, cloned into a suitable expression vector. RAK is capable of complementing the potassium-dependent phenotype of *Saccharomyces cerevisiae* strain CY162 on medium containing low potassium concentration.

The potassium channel subclass of the present invention is characterized in that the potassium channels have four hydrophobic domains capable of forming transmembrane helices. These channels are further characterized in that they comprise two pore-forming domains, one of which is interposed between said first helix and said second helix, and the other of which is interposed between said third helix and said fourth helix. The pore-forming domains

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further contain a potassium selective motif which serves to confer upon the channel the ability to pass potassium ions to the exclusion of other ions, such as sodium, calcium, and the like. In certain preferred embodiments, this motif contains the peptide Y/G, and particularly in either a dipeptide or tripeptide motif, and frequently with Y/F-G bonding. In most preferred embodiments, the motif is selected from the group consisting of G-V-G, G-L-G, G-Y-G, G-F-G, and G-I-G.

In certain embodiments of the present invention, the potassium channel is positioned within a cell membrane in such a manner as to allow it to function as a modulator of the flow of potassium ions into and out of the cell. To best regulate this activity, at least one pore-forming domain may be positioned proximal to a exterior portion of the cell membrane.

In other embodiments, the potassium channels of the present invention further comprise an amino-terminal glycosylation site, and especially wherein that site is asparagine-linked.

Potassium channels belonging to the subclass as presented herein may be derived from a wide variety of animal species, both vertebrate and invertebrate. Using the yeast expression technology and other teachings as set forth herein, the present inventors have isolated a single 2463 base pair cDNA fragment from an invertebrate source, designated Dm ORF1 [SEQ ID NO: 1], by complementation of the potassium-dependent phenotype of *Saccharomyces cerevisiae* strain CY162 (*trk1Δ*) on medium containing low potassium concentration [J.A Anderson, S.S. Huprikar, L.V. Kochian, W.J. Lucas, R.F. Gaber, *Proc. Natl. Acad. Sci USA* 89, 3736-3740 (1992)]. Dm ORF1 contains a single long open reading frame encoding a protein of 618 amino acids [SEQ ID NO:2] that exhibits substantial amino acid identity to the pore-

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forming regions of other potassium channels. The DmORF1 contains structural features that distinguish it from other classes of potassium channels, including four hydrophobic domains capable of forming transmembrane helices (M1-M4) and two putative pore forming H5 domains found between transmembrane helices M1 and M2, and M3 and M4. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity [L. Heginbotham, T. Abramson, R. MacKinnon, *Science* 258, 1152-1155, (1992)]. This work was expanded to clone a construct derived from *C. elegans* having a single open reading frame sufficient to encode a protein of 434 amino acids, designated pCORK.

A search of the GENBANK database for DNA and protein sequences similar to DmORF1 revealed several cloned potassium channel sequences including a putative protein coding DNA sequence, F22b7.7, reported in the *Caenorhabditis elegans* genome sequencing project [R. Wilson, R. Ainscough, K Anderson, et al. *Nature* 368, 32-38 (1994)]. The DNA sequence contained a single long open reading frame sufficient to encode a protein of 336 amino acids (predicted MW 38.5 kDa) with substantial homology to known potassium channel sequences.

Using the hybridization approach, a cDNA sequence designated CeORF1 [SEQ ID NO: 38] was isolated by probing a *Caenorhabditis elegans* cDNA library with oligonucleotides designed using F22b7.7 DNA sequences [T.N. Davis and J. Thorner *Meth. Enzymol.* 139, 246-262 (1987)]. CeORF1 contains a single long open reading frame encoding a protein that exhibits substantial amino acid identity to pore-forming regions of other potassium channels.

CeORF1 and pCORK each contain structural features similar to DmORF1, including two putative pore forming H5 domains. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity [L.

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Heginbotham, T. Abramson, R. MacKinnon, *Science* 258, 1152-1155, (1992)]. These features form the basis of the designation of a new sub-family of potassium channels comprising DmORF1, CORK, and CeORF1.

5 Other aspects of the present invention relate to methods of modulating potassium channel activity, by affecting the ability of such channel to allow the flow of ions into, through, or out of a cellular membrane, and particularly when these ions are potassium ions. Certain
10 substances whether biological or chemical in nature, may be applied to cell membranes having as an integral part of their structure, one or more potassium channels comprising the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ
15 ID NO: 36, or RAK, in an amount and for a time sufficient to affect the ability of the potassium channel to so regulate the flow of ions. Substances that are potassium channel blockers will inhibit the ability of the channel to regulate the flow of such ions. Substances that enhance such ability may be considered potassium channel "activators."
20 Substances that modulate the activity of RAK may do so by modulation of cardiac action potential, upward or downward.

 Application of such substances may take the form of *in vitro*, *ex vivo*, or *in vivo* application, each in a formulation suitable to deliver the substance to the cell
25 membrane and to sustain such delivery for a time sufficient to allow the substance to interact with the membrane. Appropriate formulations, concentrations of substances, application time, and other relevant parameters may be established by utilizing, *inter alia*, known assays for
30 measuring ion channel current flow. Another suitable endpoint one skilled in the art may utilize in optimizing these parameters, especially in the case of potassium channel blockers, is "cell death". Such assays may be performed *in vitro* and extrapolated to *in vivo* conditions,

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or in some cases may be easily established directly in vivo, as for example, by applying the substance directly to a test sample comprising the target insect pest (whole organism) and noting the appropriate parameters at which an acceptable per cent of insect death is attained.

In certain preferred embodiments, methods of selectively inhibiting insect pests are presented by applying to such insect pests a substance capable of selectively inhibiting the activity of a potassium channel contained in the cells of such insect, and comprising the amino acid sequence of SEQ ID NO:2, or a potassium channel substantially homologous thereto. In the most preferred embodiments, the inhibitor will inhibit the activity of the aforementioned potassium channel without inhibition of other, non-homologous potassium channels that may be present in species other than the targeted insect pest. It is envisioned that such other species may also be present at the site of application of the inhibitor, such as in a garden, crop, or other site wherein it is desired to control insect pests. In other preferred embodiments, methods of selectively inhibiting nematode pests are presented much in the same manner as discussed for control of insect pests, by applying to such pests a substance capable of selectively inhibiting the activity of a potassium channel contained in the cells of such pest, and comprising the the amino acid sequence of SEQ ID NO:4 or SEQ ID NO: 36, or potassium channels substantially homologous thereto.

The following Examples are provided to further illustrate various aspects of the present invention. They are not to be construed as limiting the invention.

Example 1

Recombinant expression library screening.

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Saccharomyces cerevisiae strain CY162 is described in Anderson, J.A. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89, 3736-3740]. Growth of bacterial strains and plasmid manipulations are performed by standard methods (Maniatis T., Molecular Cloning. Cold Spring Harbor Laboratory Press, 1982). Media conditions for growth of yeast, isolation of plasmid DNA from yeast, and DNA-mediated transformation of yeast strains are as described (Rose M. D., Methods in yeast genetics, Cold Spring Harbor Laboratory Press, 1990). A multifunctional expression library constructed in pYES2 and containing cDNA made from 3rd instar male *Drosophila melanogaster* mRNA is used as described [S.J. Elledge, J.T. Mulligan, S.W. Ramer, M. Spottswood, R.W. Davis *Proc. Natl. Acad. Sci USA* 88, 1731-1735 (1991)]. A multifunctional expression library constructed in pYES2 and containing cDNA made from mRNA obtained from all life stages of *Caenorhabditis elegans* is custom-made by Invitrogen Corporation.

Isolation of expression plasmids encoding heterologous potassium channels. CY162 cells are transformed with plasmid DNA from each library to give 3×10^6 transformants from each library on SCD-ura (synthetic complete dextrose (2 %) medium containing all necessary nutritional supplements except uracil) containing 0.1 M KCl agar medium. Transformants are replica-plated to SCG-ura (synthetic complete galactose (2 %) medium containing all necessary nutritional supplements except uracil) agar medium. Colonies that grow on this selective agar medium are transferred to SCG-ura agar medium to obtain single colonies clones and while reassaying suppression of the potassium-dependent phenotype. Plasmid DNA is isolated from surviving colonies and used to transform CY162. Six individual transformant strains containing one plasmid, pDmORF1, that confers the potassium independent phenotype is cultured on

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SCD-ura and SCG-ura medium along with CY162 strains bearing pKAT1, which encodes a plant inward rectifier potassium channel that supports the growth of CY162 on selective medium (FIGURE 1). The plasmid bearing strains exhibit
5 potassium-independent growth on both dextrose and galactose containing medium. Growth on dextrose is likely due to basal level of transcription leading to sufficient potassium channel expression to support growth.

10 Example 2

DNA sequence analysis of DmORF1. Plasmids that confer
suppression of the potassium-dependent phenotype are
subjected to automated DNA sequence analysis performed by
15 high temperature cycle sequencing (Applied Biosystems). Geneworks DNA sequence analysis software (Intelligenetics) is used to align raw DNA sequence information and to identify open reading frames. The DNA sequence of the 2.4 kb insert in pDmORF1 is displayed in FIGURE 2A and 2B [SEQ
20 ID NO:1]. The 5' untranslated sequences of the cDNA contain long poly A and poly T tracts not likely to be found in protein coding regions. The first ATG proximal to the 5' end is present in a consensus *Drosophila melanogaster* translational initiation site [D.R. Cavener *Nucleic Acids Res.*, 15, 1353-1361 (1987)], consistent with the designation
25 of this site as the translational start site. A single long open reading frame sufficient to encode a protein of 618 amino acids (predicted MW 68 kDa) is encoded in pDmORF1. A consensus polyadenylation site, AATCAA, occurs at position
30 2093-2098 in 3' untranslated sequences. The DmORF1 contains structural features that distinguish it from other classes of potassium channels, including four hydrophobic domains capable of forming transmembrane helices (M1-M4) and two pore forming H5 domains found between transmembrane helices

- 20 -

M1 and M2, and M3 and M4. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity [L. Heginbotham, T. Abramson, R. MacKinnon, *Science* 258, 1152-1155, (1992)].

5

Example 3

Identification of *Caenorhabditis elegans* sequences

10 homologous to DmORF1. A search of the GENBANK database protein sequences similar to DmORF1 reveals significant matches with several known potassium channel sequences. The
15 closest match is to a putative protein coding DNA sequence,
F22b7.7, reported in the *Caenorhabditis elegans* genome sequencing project [R. Wilson, R. Ainscough, K. Anderson, et al., *Nature* 368, 32-38 (1994)]. The DNA sequence and predicted amino acid sequence assembled from putative exons recognized by a GENBANK exon identification algorithm is displayed in FIGURE 3A and 3B [SEQ ID NOS:3 and 4]. The DNA
20 sequence contains a single long open reading frame sufficient to encode a protein of 336 amino acids (predicted MW 38.5 kDa) with substantial homology to known potassium channel sequences. The F22b7.7 sequence contains structural features that distinguish it from other classes of potassium
25 channels, including three of four hydrophobic domains capable of forming transmembrane helices (M1-M4) identified in DmORF1 and two pore forming H5 domains found between transmembrane helices a predicted M1 and M2, and M3 and M4. Each pore forming H5 domain contains the Y/F-G dipeptide
30 motif required for potassium selectivity [L. Heginbotham, T. Abramson, R. MacKinnon, *Science* 258, 1152-1155, (1992)]. The lack of an amino terminal transmembrane domain homologous to DmORF1 M1 in the F22b7.7 sequence may be due to failure of the search algorithm to identify exon(s)

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encoding the amino terminus. Alternatively, an amino terminal coding sequence may be added by trans-splicing, which occurs frequently in *Caenorhabditis elegans*.

5

Example 4

Cloning and DNA sequence analysis of CeORF1.

Oligonucleotides corresponding to DNA sequences encoding the two pore forming domains of F22b7.7 are synthesized using an Applied Biosystems DNA synthesizer.

10

F22b7.7-H2-1:

5'TCCATTTTCTTTGCCGTAACCGTCGTCACCTACCATCGGATACGGTAATCCA [SEQ ID NO:5]. F22b7.7-H2-2:

15

5'TCATTCTACTGGTCCTTCATTACAATGACTACTGTCGGGTTTGGCGACTTG [SEQ ID NO:6]. The oligos were labelled at their 5' ends with ³²P using a 5'-end labelling kit according to manufacturers instructions (New England Nuclear). The labelled oligos are pooled and used to screen 6 x 10⁵ plaques from a λZAP-*Caenorhabditis elegans* cDNA library (obtained from Clontech) by published methods [T.N. Davis and J. Thorner *Meth. Enzymol.* 139, 246-262 (1987)]. Hybridization is at 42°C for 16 hours. Positive clones are plaque-purified by twice repeating the hybridization screening process.

20

Plasmid DNAs, excised from phage DNA according to manufacturers instructions, are subjected to automated DNA sequence analysis performed by high temperature cycle sequencing (Applied Biosystems). Geneworks DNA sequence analysis software (Intelligenetics) is used to align raw DNA sequence data and to identify open reading frames.

25

30

Example 5

Comparison of the putative proteins encoded by DmORF1 and F22b7.7. Predicted amino acid sequences of DmORF1 and

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F22b7.7 are aligned and displayed in FIGURE 4 [SEQ ID NOS:37 and 38]. Only limited overall amino acid homology is exhibited by these two proteins with regions of greatest homology existing in the pore forming H2-1 and H2-2 domains.

5 FIGURE 5A shows a comparison of the pore forming domains of DmORF1 and F22b7.7 with those of the known *Drosophila melanogaster* potassium channel and inward rectifier sequences [SEQ ID NOS:7 through 21]. Amino acid identities greater than 50 % are observed with all potassium channel

10 sequences. FIGURE 5B shows hydropathy plot analysis of DmORF1 and F22b7.7. The two proteins, which show remarkable topological similiarity through their length, are predicted to be composed of four membrane-spanning hydrophobic domains (M1-M4), and two pore forming H2 domains. These data

15 suggest the predicted topology shown in FIGURE 6. Both proteins are predicted to span the membrane four times with amino and carboxyl termini residing within the cell. This topology places the single amino-terminal asparagine-linked glycosylation site and H2 domains on the cell exterior

20 permitting permeation of the membrane by the pore forming domains from the outside, an absolute requirement for the formation of a functional potassium channel.

Example 6

25 Functional expression of a rat atrial delayed rectifier potassium channel in yeast. CY162 transformants containing plasmids pKAT1, which encodes a plant inward rectifier potassium channel, pRATRAK, which encodes a rat atrial delayed rectifier potassium channel, pDmORF1, and control

30 plasmid pYES are cultured on arginine-phosphate-dextrose agar medium lacking ura medium [A. Rodriguez-Navarro and J. Ramos, *J. Bacteriol.* 159, 940-945, (1984)] containing various KCl concentrations (FIGURE 7). Strains containing pKAT1, pRATRAK, and pDmORF1 all support the growth of CY162

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on medium containing a low concentration of potassium, while pYES2 containing CY162 cells only grow on medium containing a high potassium concentration, indicating that heterologous potassium channels of several different types function to provide high affinity potassium uptake.

pRATRAK is constructed by modifying the protein-coding sequences of RATRAK to add 5' HindIII and 3' XbaI sites using PCR. In addition, four A residues are added to the sequences immediately 5' proximal to the initiator ATG to provide a good yeast translational initiation site. The modified fragment is cloned into the HindIII and XbaI sites in the yeast expression vector pYES2 (Invitrogen), forming pRATRAK.

Example 7

Bioassay of functional expression of heterologous potassium channels

Yeast strains dependent on heterologous potassium channels for growth should be sensitive to non-specific potassium channel blocking compounds. To test the potassium channel blocking properties of several compounds, a convenient agar plate bioassay is employed. Strains containing pKAT1, pRATRAK, pDmORF1, and pYES2 are plated in arginine-phosphate-dextrose agar medium lacking ura and containing various amounts of potassium chloride. Arginine-phosphate-dextrose medium is used to avoid interference from potassium and ammonium ions present in standard synthetic yeast culture medium. Sterile filter disks were placed on the surface of the agar and saturated with potassium channel blocking ions CsCl, BaCl₂, and TEA. The growth of heterologous potassium channel containing strains is inhibited by potassium channel blocking ions, in a channel dependent manner. DmORF1-dependent growth is blocked by

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BaCl₂ but not by CsCl or TEA. KAT-dependent growth is blocked by BaCl₂, CsCl and TEA. RATRAK-dependent growth is blocked by BaCl₂, CsCl and TEA to a much greater extent than pKAT1, reflecting in part a slower growth rate of pRATRAK-containing cells. These observations confirm that these channels support the growth of the mutant yeast cells and demonstrate the efficacy of the yeast bioassay for screening for compounds that block potassium channel function. The control pYES-containing strain grows only around applied KCl and RbCl, a congener of KCl.

Example 8

Identification of compounds that alter potassium channel activity

Yeast strains made capable of growing on medium containing low potassium concentration by expression of heterologous potassium channels are used to screen libraries of chemical compounds of diverse structure for those that interfere with channel function. CY162 cells containing pKAT1, pRATRAK, pDmORF1, pCeORF1, and pYES2-TRK1 (10⁴/ml) are plated in 200 ml of arginine-phosphate-dextrose agar medium lacking ura and containing 0.2 mM potassium chloride in 500 cm² plates. The CY162 cells bearing pYES2-TRK1 are included in the assay as a control to identify compounds that have non-specific effects on the yeast strain and are therefore not specifically active against the heterologous potassium channels. Samples of chemical compounds of diverse structure (2 µl of 10 mg/ml solution in DMSO) are applied to the surface of the hardened agar medium in a 24 x 24 array. The plates are incubated for 2 days at 30°C during which time the applied compounds radially diffuse into the agar medium. The effects of applied compounds on strains bearing heterologous potassium channel

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genes are compared to the pYES2-*TRK1* bearing strain. Compounds that cause a zone of growth inhibition around the point of application that is larger on plates containing cells bearing the heterologous potassium channels than that observed around the pYES2-*TRK1* bearing strains are considered selective potassium channel blockers. Compounds that induce a zone of enhanced growth around the point of application that is larger on plates containing cells bearing the heterologous potassium channels than that observed around the pYES2-*TRK1* bearing strains are considered selective potassium channel openers.

Example 9

DmORF1-induced currents in *X. laevis* oocytes assayed by two-electrode voltage clamp

DNA sequence analysis of the pDmORF insert strongly suggest that the protein encoded by the single long ORF possesses properties in common with known potassium channels. To test this hypothesis, the electrophysiological properties of the putative potassium channel encoded by DmORF was examined by expression in *X. laevis* oocytes. Currents were measured by two-electrode whole-cell voltage clamp. DNA sequences encoding the open reading frame of DmORF1 were amplified by polymerase chain reaction (PCR) using the following oligonucleotides:

MPO23: ATAAAGCTTAAAAATGTCGCCGAATCGATGGAT [SEQ ID NO:22]

MPO24: AGCTCTAGACCTCCATCTGGAAGCCCATGT [SEQ ID NO:23]

The full length PCR product was cloned into corresponding sites in pSP64 poly A (Promega), forming pMP147. Template DNA was linearized with EcoRI and RNA transcribed using the Message Machine (Ambion) *in vitro* transcription kit according to manufacturers instructions. A sample of the RNA was resolved in a MOPS-acetate-formaldehyde agarose gel

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and RNA content was estimated by ethidium bromide staining. The remainder was stored on dry ice. *X. laevis* oocytes were isolated and injected with 50 nl of sterile TE containing 5-20 ng transcript according to published procedures. After three days, whole oocyte currents were recorded using a two-electrode voltage clamp. Electrodes contained 3M KCl and had resistances of 0.3-1.0 MΩ. Recordings were performed with constant perfusion at room temperature in the presence of either low (10 mM) or high (90 mM) potassium chloride. Two electrode voltage clamp analysis of the DmORF1 gene product expressed in *X. laevis* oocytes demonstrates properties of a voltage- and potassium-dependent potassium channel. At low potassium concentrations, DmORF1 exhibited outward current at depolarizing potentials. At high potassium concentration, DmORF1 exhibits both inward and outward currents. The DmORF1 channel displays a high preference for potassium and shows cation selectivity in the rank order K>Rb>NH₄>Cs>Na>Li. Potassium currents were greatly attenuated by BaCl₂.

Example 10

Developmental regulation of DmORF1 expression in *D. melanogaster* determined by northern blotting analysis

Isolation of pDmORF1 from a *D. melanogaster* expression library strongly suggests that the insert contained within originated in mRNA from that species. Detailed understanding of the developmental regulation of DmORF1 expression should aid in determining strategies for use of DmORF1 as a target for novel insecticides. To characterize DmORF1 expression, northern blotting analysis of poly A RNA from various stages of the *D. melanogaster* life cycle was carried out.

D. melanogaster poly A⁺ RNA from embryo, larvae and

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adult forms (Invitrogen, 5 µg) was resolved in a MOPS-acetate-formaldehyde agarose gel according to standard procedures. The gel was stained with ethidium bromide and photographed to mark the positions of 18 S and 28 S ribosomal RNAs used as molecular weight markers. RNA was transferred by capillary action to nitrocellulose with 10 x SSPE. The blot was air-dried, baked for one hour at 80°C, and prehybridized in 4x SSPE, 1% SDS, 2x Denhardt's, 0.1 % single stranded DNA at 68 °C for 2 hours.

A 2.4 kb XhoI fragment of DmORF1 was isolated from pDmORF1 and labeled with α -³²P dCTP using the Ready-to-Go kit (Pharmacia) according to manufacturers instructions. The probe was denatured by heating to 100°C for 5 minutes followed by quenching in an ice water bath. The probe was added to the prehybridization solution and hybridization continued for 24 hours at 68 °C.

The blot was washed briefly with 2x SSPE, 0.1% SDS at room temperature followed by 0.5 x SSPE, 0.1 % SDS at 65 °C for 2 hours. The blot was air-dried and exposed to Reflection X-ray film (NEN) using an intensifying screen at -70 °C for 48 hours.

Northern blotting analysis indicates that the DmORF1 probe hybridizes to an mRNA species of approximately 2.8 kb isolated from *D. melanogaster* embryo, larvae, and adult forms. The length of the DmORF1 mRNA corresponds well with the length of the predicted ORF. Thus, the DmORF is expressed at all developmental stages in the life cycle of *D. melanogaster*.

Example 11

Expression of the DmORF1 gene product in vitro.

DNA sequence analysis of the pDmORF1 insert reveals a single long ORF with conserved amino acid sequence domains

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in common with known potassium channels. The DNA sequence predicts an ORF sufficient to encode a protein of 618 amino acid in length. The DmORF1 polypeptide contains four segments of at least 20 hydrophobic amino acids in length suggesting that the segments span the plasma membrane. In addition, the DmORF1 protein sequence contains a putative N-linked glycosylation site (Asn-Thr-Thr) at amino acids 58-60. To confirm that a protein of the predicted size of DmORF is expressed from the insert in pDmORF1 and to test the proposition that DmORF1 is glycosylated, pDmORF1 was used as template to drive coupled in vitro transcription/translation.

Plasmid pMP147 was used as template to produce ³⁵S-labeled DmORF gene product in vitro using a TnT coupled transcription-translation kit (Promega) according to manufacturers instructions. Glycosylation of the nascent DmORF1 polypeptide was accomplished by addition of canine pancreatic microsomes (Promega) to the transcription-translation reaction. Samples of glycosylated DmORF protein were treated with endoglycosidase H to remove added carbohydrate moieties. Aliquots were precipitated with TCA and collected on GF/C filters, washed with ethanol, dried and counted. Equivalent cpm's were resolved by SDS-PAGE. The gel was impregnated with soluble fluor Amplify (Amersham) and dried onto Whatman 3MM paper. The dried gel was exposed to Reflection X-ray film at room temperature.

Translation of the DmORF1 gene product in vitro produced a polypeptide of 68 kDa, consistent with the predicted molecular weight of the ORF. Translation of DmORF1 in the presence of canine pancreatic microsomes results in synthesis of a protein with reduced electrophoretic mobility, consistent with glycosylation of the nascent polypeptide. Treatment of glycosylated DmORF with EndoH increased its relative mobility as expected upon

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removal of carbohydrate moieties. Thus, the pDmORF1 insert is capable of directing the expression of a glycoprotein with the expected molecular weight. EndoH treatment removes carbohydrate residues consistent with the sugar added through N-linked glycosylation.

Example 12

High-affinity K⁺ uptake and selectivity of DmORF1 expressed in yeast.

Expression of DmORF permits CY162 cells to grow on medium containing a low concentration of potassium, implying that DmORF1 supplies high affinity potassium uptake capacity. To characterize the potassium uptake properties of CY162 cells containing DmORF1, ⁸⁶Rb uptake studies were performed. Examination of the uptake of this potassium congener revealed important aspects of potassium uptake by DmORF1.

Yeast strains containing heterologous potassium-expression plasmids CY162-DmORF1, CY162-pKAT and the control strain CY162-pYES2 (Clontech) were cultured overnight in SC Gal-ura containing 0.1 M KCl. The cells were harvested, washed with sterile doubled distilled water and starved for K⁺ for 6 hours in Ca-MES buffer. Cells were washed again and distributed to culture tubes (10⁸ cells/tube) containing ⁸⁶RbCl in Ca-MES buffer. The tubes were incubated at room temperature, samples filtered at various time intervals and counted. ⁸⁶Rb uptake into cells was displayed. For Double Reciprocal analysis, ⁸⁶Rb was held constant and barium ions varied to determine K_i values.

The high-affinity potassium uptake capacity encoded by DmORF1 permits high-affinity uptake of the potassium congener, ⁸⁶Rb, as well. Barium inhibited ⁸⁶Rb uptake with a

- 30 -

Ki of μM as demonstrated in Double Reciprocal analysis. No high affinity ^{86}Rb uptake is observed in control CY162-pYES2 cells and ^{86}Rb uptake into CY162-pKAT cells is consistent with its published properties.

5

Example 13Expression of *Drosophila melanogaster* potassium channels in yeast.

10 Voltage-gated potassium channel diversity in the fruitfly *Drosophila melanogaster* is encoded in large part by six genes, Shaker, Shab, Shal, Shaw, Eag, and Slo. Expression of these potassium channels in yeast will permit their introduction into screening assays for novel
15 insecticidal compounds and facilitate characterization of their ion channel properties and sensitivity to compounds with activating and inhibitory properties.

DNA sequences encoding *Drosophila melanogaster* potassium channels were amplified by PCR using synthetic
20 oligonucleotides that add 5' HindIII or Kpn I, sites and 3' XbaI, SphI, or XhoI sites:

Shaker 5':AAAAAGCTTAAAATGGCACACATCACG [SEQ ID NO:24]

Shaker 3':AAACTCGAGTCATACCTGTGGACT [SEQ ID NO:25]

25

Shab 5':AAAAAGCTTAAAATGGTCGGGCAATTG [SEQ ID NO:26]

Shab 3':AAAAGCATGCTCATCTGGATGGGCA [SEQ ID NO:27]

Shal 5':AAAAAGCTTAAAATGGCCTCGGTCGCC [SEQ ID NO:28]

30

Shal 3':TTTCTAGACTACATCGTTGTCTT [SEQ ID NO:29]

Shaw 5':AAAAAGCTTAAAATGAATCTGATCAAC [SEQ ID NO:30]

Shaw 3':AAATCTAGATTAGTCGAAACTGAA [SEQ ID NO:31]

- 31 -

Eag 5':AAAAAGCTTAAATGCCTGGCGGA [SEQ ID NO:32]

Eag 3':AAATCTAGAGGCTACAGGAAGTCC [SEQ ID NO:33]

Slo 5':GGGGGTACCAAATGTCGGGGTGTGAT [SEQ ID NO:34]

5 Slo 3':TTTTTCTAGATCAAGAGTTATCATC [SEQ ID NO:35]

Plasmids used as templates for the PCR reactions were:
 pBSc-DShakerH37, pBSc-dShab11, pBSc-dShal2+(A)₃₆, pBScMXT-
 dShaw [A. Wei, M. Covarrubias, A. Butler, K. Baker, M. Pak,
 10 L. Salkoff, *Science* 248, 599-603 (1990), provided by L.
 Salkoff], pBScMXT-slo,v4 [N.S. Atkinson, G.A. Robertson, B.
 Ganetzky, *Science* 253,551-555, (1991), provided by L.
 Salkoff], and pBIMCH20 Eag [CH20] [J. Warmke, R. Drysdale,
 B. Ganetzky, *Science* 252, 1560-1564 (1991), A. Bruggemann,
 15 L.A. Pardo, W. Stuhmer, O. Pongs, *Nature* 365, 445-448
 (1993), provided by B. Ganetzky].

Amplified fragments were digested with the appropriate
 restriction endonucleases, purified using GeneClean (Bio
 101), and ligated into corresponding sites in pYES2
 20 (Invitrogen). CY162 cells were transformed with assembled
Drosophila melanogaster potassium channel expression
 plasmids by the LiCl method and plated on SCD-ura containing
 0.1M KCl agar medium. Selected transformants were tested
 for growth on arginine-phosphate-galactose (2 %)/sucrose
 25 (0.2 %)-ura agar medium containing 1-5 mM KCl. CY162 cells
 containing pKAT1 or pDmORF1 were cultured as positive
 controls and CY162 cells containing pYES2 were grown to
 provide a negative control.

CY162 cells bearing *Drosophila melanogaster* potassium
 30 channel expression plasmids survive under conditions in
 which growth is dependent on functional potassium channel
 expression. At potassium ion concentrations between 1-3 mM,
 negative control CY162 cells containing pYES2 grow poorly.
 Expression of the *Drosophila melanogaster* potassium channels

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Shal, Shaw and Eag substantially improve growth of CY162. These results are consistent with the *Drosophila melanogaster* potassium channels providing high-affinity potassium uptake capacity. This capacity is apparently sufficient to replace the native high-affinity potassium transport capacity encoded by TRK1 which is lacking in CY162 (*trk1 trk2*) cells.

Example 14

Cloning of a novel *C. elegans* sequence with homology to potassium channels.

In order to expand the applicability of this technology to discover compounds with novel anhelmenthic activity, CY162 cells were transformed with a pYES2-based yeast expression library constructed using cDNA synthesized from *C. elegans* mRNA (Invitrogen). Plasmid DNA isolated from yeast cells that survived the selection scheme described in EXAMPLE 1 were subjected to automated DNA sequence analysis performed by high temperature cycle sequencing (Applied Biosystems). Geneworks DNA sequence analysis software (Intelligenetics) is used to align raw DNA sequence information and to identify open reading frames. The DNA sequence of the 1.4 kb insert in pCORK is displayed in FIGURE 9A and 9B. The 5' untranslated sequences of the cDNA are present in this construct. A single long open reading frame sufficient to encode a protein of 434 amino acids (predicted MW 48 kDa) is predicted in pCORK [SEQ ID NO:38]. A consensus polyadenylation site, AATAAA, occurs at position 1359-1364 in 3' untranslated sequences and is followed by a tract of 15 consecutive A residues. The CORK ORF contains structural features that resemble pore forming H5 domains found in potassium channels. Two putative pore forming H5 domains (residues 76-39 and 150-162) contain the G-Y/F-G

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tripeptide motif required for potassium selectivity [L. Heginbotham, T. Abramson, R. MacKinnon, Science 258, 1152-1155, (1992)].

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: American Cyanamid Company
- (ii) TITLE OF INVENTION: Genes Encoding a Novel Family of Potassium Channels
- (iii) NUMBER OF SEQUENCES: 38
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: American Cyanamid Company
 - (B) STREET: One Cyanamid Plaza
 - (C) CITY: Wayne
 - (D) STATE: New Jersey
 - (E) COUNTRY: USA
 - (F) ZIP: 07470-8426
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Matthews, Gale F.
 - (B) REGISTRATION NUMBER: 32,369
 - (C) REFERENCE/DOCKET NUMBER: 32,421-01 PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 201-660-6329
 - (B) TELEFAX: 201-660-7160

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2441 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 190..2043

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACGCGATCGC CGCGAGTGTA TATTTTTTTTTT TTAGCTCAGT CTTTCAGTGTT TCGCGATTCT	60
CTTTAAAAGA AAAAAAAAAAT AATAAGTCAA AACTACAAAC CACACAGCGA AAGGCGAAAG	120
CAACGGTTCC TGCGAGTGTT TATTTTTTTTTT TTCAACAATT TTTGATCGTA GTGCGACAAT	180
CCGTCGAGC ATG TCG CCG AAT CGA TGG ATC CTG CTG CTC ATC TTC TAC	228
Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr	
1 5 10	
ATA TCC TAC CTG ATG TTC GGG GCG GCA ATC TAT TAC CAT ATT GAG CAC	276
Ile Ser Tyr Leu Met Phe Gly Ala Ala Ile Tyr Tyr His Ile Glu His	

35

15	20	25	
GGC GAG GAG AAG ATA TCG CGC GCC GAA CAG CGC AAG GCG CAA ATT GCA Gly Glu Glu Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Gln Ile Ala 30 35 40 45			324
ATC AAC GAA TAT CTG CTG GAG GAG CTG GGC GAC AAG AAT ACG ACC ACA Ile Asn Glu Tyr Leu Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr 50 55 60			372
CAG GAT GAG ATT CTT CAA CGG ATC TCG GAT TAC TGT GAC AAA CCG GTT Gln Asp Glu Ile Leu Gln Arg Ile Ser Asp Tyr Cys Asp Lys Pro Val 65 70 75			420
ACA TTG CCG CCG ACA TAT GAT GAT ACG CCC TAC ACG TGG ACC TTC TAC Thr Leu Pro Pro Thr Tyr Asp Asp Thr Pro Tyr Thr Trp Thr Phe Tyr 80 85 90			468
CAT GCC TTC TTC TTC GCC TTC ACC GTT TGC TCC ACG GTG GGA TAT GGG His Ala Phe Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly 95 100 105			516
AAT ATA TCG CCA ACC ACC TTC GCC GGA CGG ATG ATC ATG ATC GCG TAT Asn Ile Ser Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr 110 115 120 125			564
TCG GTG ATT GGC ATC CCC GTC AAT GGT ATC CTC TTT GCC GGC CTC GGC Ser Val Ile Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly 130 135 140			612
GAA TAC TTT GGA CGT ACG TTT GAA GCG ATC TAC AGA CGC TAC AAA AAG Glu Tyr Phe Gly Arg Thr Phe Glu Ala Ile Tyr Arg Arg Tyr Lys Lys 145 150 155			660
TAC AAG ATG TCC ACG GAT ATG CAC TAT GTC CCG CCG CAG CTG GGA TTG Tyr Lys Met Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu 160 165 170			708
ATC ACC ACG GTG GTG ATT GCC CTG ATT CCG GGA ATA GCT CTC TTC CTG Ile Thr Thr Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu 175 180 185			756
GTG CTG CCC TGC GTG GGT GTT CAC CTA CTT CGA GAA CTG GGC CTA TCT Val Leu Pro Cys Val Gly Val His Leu Leu Arg Glu Leu Gly Leu Ser 190 195 200 205			804
TCC ATC TCG CTG TAC TAC AGC TAT GTG ACC ACC ACA ACA ATT GGA TTC Ser Ile Ser Leu Tyr Tyr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe 210 215 220			852
GGT GAC TAT GTG CCC ACA TTT GGA GCC AAC CAG CCC AAG GAG TTC GGC Gly Asp Tyr Val Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Gly 225 230 235			900
GGC TGG TTC GTG GTC TAT CAG ATC TTT GTG ATC GTG TGG TTC ATC TTC Gly Trp Phe Val Val Tyr Gln Ile Phe Val Ile Val Trp Phe Ile Phe 240 245 250			948
TCG CTG GGA TAT CTT GTG ATG ATC ATG ACA TTT ATC ACT CGG GGC CTC Ser Leu Gly Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu 255 260 265			996
CAG AGC AAG AAG CTG GCA TAC CTG GAG CAG CAG TTG TCC TCC AAC CTG Gln Ser Lys Lys Leu Ala Tyr Leu Glu Gln Gln Leu Ser Ser Asn Leu 270 275 280 285			1044
AAG GCC ACA CAG AAT CGC ATC TGG TCT GGC GTC ACC AAG GAT GTG GGC Lys Ala Thr Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val Gly 290 295 300			1092

TAC CTC CGG CGA ATG CTC AAC GAG CTG TAC ATC CTC AAA GTG AAG CCT Tyr Leu Arg Arg Met Leu Asn Glu Leu Tyr Ile Leu Lys Val Lys Pro 305 310 315	1140
GTG TAC ACC GAT GTA GAT ATC GCC TAC ACA CTG CCA CGT TCC AAT TCG Val Tyr Thr Asp Val Asp Ile Ala Tyr Thr Leu Pro Arg Ser Asn Ser 320 325 330	1188
TGT CCG GAT CTG AGC ATG TAC CGC GTG GAG CCG GCT CCC ATT CCC AGC Cys Pro Asp Leu Ser Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser 335 340 345	1236
CGG AAG AGG GCA TTC TCC GTG TGC GCC GAC ATG GTT GGC GCC CAA AGG Arg Lys Arg Ala Phe Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg 350 355 360 365	1284
GAG GCG GGC ATG GTA CAC GCC AAT TCC GAT ACG GAT CTA ACC AAA CTG Glu Ala Gly Met Val His Ala Asn Ser Asp Thr Asp Leu Thr Lys Leu 370 375 380	1332
GAT CGC GAG AAG ACA TTC GAG ACG GCG GAG GCG TAC CAC CAG ACC ACC Asp Arg Glu Lys Thr Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr 385 390 395	1380
GAT TTG CTG GCC AAG GTG GTC AAC GCA CTG GCC ACG GTG AAG CCA CCG Asp Leu Leu Ala Lys Val Val Asn Ala Leu Ala Thr Val Lys Pro Pro 400 405 410	1428
CCG GCG GAA CAG GAA GAT GCG GCT CTC TAT GGT GGC TAT CAT GGC TTC Pro Ala Glu Gln Glu Asp Ala Ala Leu Tyr Gly Gly Tyr His Gly Phe 415 420 425	1476
TCC GAC TCC CAG ATC CTG GCC AGC GAA TGG TCG TTC TCG ACG GTC AAC Ser Asp Ser Gln Ile Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn 430 435 440 445	1524
GAG TTC ACA TCA CCG CGA CGT CCA AGA GCA CGT GCC TGC TCC GAT TTC Glu Phe Thr Ser Pro Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe 450 455 460	1572
AAT CTG GAG GCA CCT CGC TGG CAG AGC GAG AGG CCA CTG CGT TCG AGC Asn Leu Glu Ala Pro Arg Trp Gln Ser Glu Arg Pro Leu Arg Ser Ser 465 470 475	1620
CAC AAC GAA TGG ACA TGG AGC GGC GAC AAC CAG CAG ATC CAG GAG GCA His Asn Glu Trp Thr Trp Ser Gly Asp Asn Gln Gln Ile Gln Glu Ala 480 485 490	1668
TTC AAC CAG CGC TAC AAG GGA CAG CAG CGT GCC AAC GGA GCA GCC AAC Phe Asn Gln Arg Tyr Lys Gly Gln Gln Arg Ala Asn Gly Ala Ala Asn 495 500 505	1716
TCG ACC ATG GTC CAT CTG GAG CCG GAT GCT TTG GAG GAG CAG CTG AGA Ser Thr Met Val His Leu Glu Pro Asp Ala Leu Glu Glu Gln Leu Arg 510 515 520 525	1764
AAC AAT CAC CGG GTG CCG GTC GCG TCA AGA AGT TCT CCA TGC CGG ATG Asn Asn His Arg Val Pro Val Ala Ser Arg Ser Ser Pro Cys Arg Met 530 535 540	1812
GTC TGC GAC GTC TGT TTC CCT TCC AGA AGA AGC ACC CCT CGC AGG ATC Val Cys Asp Val Cys Phe Pro Ser Arg Arg Ser Thr Pro Arg Arg Ile 545 550 555	1860
TGG AGC GCA AGT TGT CCG TGG TCT CGG TAC CCG AGG GTG TCA TCT CGC Trp Ser Ala Ser Cys Pro Trp Ser Arg Tyr Pro Arg Val Ser Ser Arg 560 565 570	1908
AGG AAG CCA GAT CCC CGC TGG ACT ACT ACA TCA ACA CGG TCA CGG CGG	1956

Arg Lys Pro Asp Pro Arg Trp Thr Thr Thr Ser Thr Arg Ser Arg Arg
 575 580 585

CCT CCA GTC AAT CCT ATT TGC GCA ACG GAC GCG GTC CGC CAC CGC CCT 2004
 Pro Pro Val Asn Pro Ile Cys Ala Thr Asp Ala Val Arg His Arg Pro
 590 595 600 605

TCG AAT CGA ATG GCA GCT TGG CCA GCG GCG GCG GCG GGC TAACGAACAT 2053
 Ser Asn Arg Met Ala Ala Trp Pro Ala Ala Ala Ala Gly
 610 615

GGGCTTCCAG ATGGAGGATG GAGCAACCCC GCCATCGGCA TTGGGCGGTG GAGCCTATCA 2113
 ACGCAAGGCG GCTGCTGGCA AGCGCCGACG CGAGAGCATC TACACCCAGA ATCAAGCCCC 2173
 ATCCGCTCGC CGGGGCAGCA TGTATCCGCC GACCGCGCAC GCCTTGGCCC AGATGCAGAT 2233
 GCGACGCGGC AGCTTGGCAA CCAGTGGCTC TGGATCGGCG GCCATGGCGG CAGTGGCCCGC 2293
 GCGTCGTGGC AGCCTCTTCC CAGCTACAGC ATCGGCATCA TCGCTGACCT CTGCTCCGCG 2353
 CCGAAGCAGC ATATTCTCGG TTACCTCCGA AAAGGATATG AATGTGCTGG AGCAGACGAC 2413
 CATTGCGGAT CTGATTCGTG CGCTCGAG 2441

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 618 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr Ile Ser Tyr
 1 5 10 15

Leu Met Phe Gly Ala Ala Ile Tyr Tyr His Ile Glu His Gly Glu Glu
 20 25 30

Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Gln Ile Ala Ile Asn Glu
 35 40 45

Tyr Leu Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr Gln Asp Glu
 50 55 60

Ile Leu Gln Arg Ile Ser Asp Tyr Cys Asp Lys Pro Val Thr Leu Pro
 65 70 75 80

Pro Thr Tyr Asp Asp Thr Pro Tyr Thr Trp Thr Phe Tyr His Ala Phe
 85 90 95

Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn Ile Ser
 100 105 110

Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr Ser Val Ile
 115 120 125

Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly Glu Tyr Phe
 130 135 140

Gly Arg Thr Phe Glu Ala Ile Tyr Arg Arg Tyr Lys Lys Tyr Lys Met
 145 150 155 160

Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu Ile Thr Thr
 165 170 175

Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu Val Leu Pro
 180 185 190
 Cys Val Gly Val His Leu Leu Arg Glu Leu Gly Leu Ser Ser Ile Ser
 195 200 205
 Leu Tyr Tyr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe Gly Asp Tyr
 210 215 220
 Val Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Gly Gly Trp Phe
 225 230 235 240
 Val Val Tyr Gln Ile Phe Val Ile Val Trp Phe Ile Phe Ser Leu Gly
 245 250 255
 Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu Gln Ser Lys
 260 265 270
 Lys Leu Ala Tyr Leu Glu Gln Gln Leu Ser Ser Asn Leu Lys Ala Thr
 275 280 285
 Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val Gly Tyr Leu Arg
 290 295 300
 Arg Met Leu Asn Glu Leu Tyr Ile Leu Lys Val Lys Pro Val Tyr Thr
 305 310 315 320
 Asp Val Asp Ile Ala Tyr Thr Leu Pro Arg Ser Asn Ser Cys Pro Asp
 325 330 335
 Leu Ser Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser Arg Lys Arg
 340 345 350
 Ala Phe Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg Glu Ala Gly
 355 360 365
 Met Val His Ala Asn Ser Asp Thr Asp Leu Thr Lys Leu Asp Arg Glu
 370 375 380
 Lys Thr Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr Asp Leu Leu
 385 390 395 400
 Ala Lys Val Val Asn Ala Leu Ala Thr Val Lys Pro Pro Pro Ala Glu
 405 410 415
 Gln Glu Asp Ala Ala Leu Tyr Gly Gly Tyr His Gly Phe Ser Asp Ser
 420 425 430
 Gln Ile Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn Glu Phe Thr
 435 440 445
 Ser Pro Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe Asn Leu Glu
 450 455 460
 Ala Pro Arg Trp Gln Ser Glu Arg Pro Leu Arg Ser Ser His Asn Glu
 465 470 475 480
 Trp Thr Trp Ser Gly Asp Asn Gln Gln Ile Gln Glu Ala Phe Asn Gln
 485 490 495
 Arg Tyr Lys Gly Gln Gln Arg Ala Asn Gly Ala Ala Asn Ser Thr Met
 500 505 510
 Val His Leu Glu Pro Asp Ala Leu Glu Glu Gln Leu Arg Asn Asn His
 515 520 525
 Arg Val Pro Val Ala Ser Arg Ser Ser Pro Cys Arg Met Val Cys Asp
 530 535 540

39

Val Cys Phe Pro Ser Arg Arg Ser Thr Pro Arg Arg Ile Trp Ser Ala
 545 550 555 560

Ser Cys Pro Trp Ser Arg Tyr Pro Arg Val Ser Ser Arg Arg Lys Pro
 565 570 575

Asp Pro Arg Trp Thr Thr Thr Ser Thr Arg Ser Arg Arg Pro Pro Val
 580 585 590

Asn Pro Ile Cys Ala Thr Asp Ala Val Arg His Arg Pro Ser Asn Arg
 595 600 605

Met Ala Ala Trp Pro Ala Ala Ala Ala Gly
 610 615

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1011 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1008

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG TCC GAT CAG CTG TTT GTC GCA TTT GAG AAG TAT TTC TTG ACG AGT	48
Met Ser Asp Gln Leu Phe Val Ala Phe Glu Lys Tyr Phe Leu Thr Ser	
1 5 10 15	
AAC GAG GTC AAG AAG AAT GCA GCA ACG GAG ACA TGG ACA TTT TCA TCG	96
Asn Glu Val Lys Lys Asn Ala Ala Thr Glu Thr Trp Thr Phe Ser Ser	
20 25 30	
TCC ATT TTC TTT GCC GTA ACC GTC GTC ACT ACC ATC GGA TAC GGT AAT	144
Ser Ile Phe Phe Ala Val Thr Val Val Thr Thr Ile Gly Tyr Gly Asn	
35 40 45	
CCA GTT CCA GTG ACA AAC ATT GGA CGG ATA TGG TGT ATA TTG TTC TCC	192
Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Cys Ile Leu Phe Ser	
50 55 60	
TTG CTT GGA ATA CCT CTA ACA CTG GTT ACC ATC GCT GAC TTG GCA GGT	240
Leu Leu Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Asp Leu Ala Gly	
65 70 75 80	
AAA TTC CTA TCT GAA CAT CTT GTT TGG TTG TAT GGA AAC TAT TTG AAA	288
Lys Phe Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys	
85 90 95	
TTA AAA TAT CTC ATA TTG TCA CGA CAT CGA AAA GAA CGG AGA GAG CAC	336
Leu Lys Tyr Leu Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His	
100 105 110	
GTT TGT GAG CAC TGT CAC AGT CAT GGA ATG GGG CAT GAT ATG AAT ATC	384
Val Cys Glu His Cys His Ser His Gly Met Gly His Asp Met Asn Ile	
115 120 125	
GAG GAG AAA AGA ATT CCT GCA TTC CTG GTA TTA GCT ATT CTG ATA GTA	432
Glu Glu Lys Arg Ile Pro Ala Phe Leu Val Leu Ala Ile Leu Ile Val	
130 135 140	
TAT ACA GCG TTT GGC GGT GTC CTA ATG TCA AAA TTA GAG CCG TGG TCT	480
Tyr Thr Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser	

145	150	155	160	
TTC TTC ACT TCA TTC TAC TGG TCC TTC ATT ACA ATG ACT ACT GTC GGG				528
Phe Phe Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly	165	170	175	
TTT GGC GAC TTG ATG CCC AGA AGG GAC GGA TAC ATG TAT ATC ATA TTG				576
Phe Gly Asp Leu Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu	180	185	190	
CTC TAT ATC ATT TTA GGT AAA TTT TCA ATG AAA AAA AAA CAA AAA TTC				624
Leu Tyr Ile Ile Leu Gly Lys Phe Ser Met Lys Lys Lys Gln Lys Phe	195	200	205	
AAA ATA TTT TTA GGT CTT GCA ATA ACT ACA ATG TGC ATT GAT TTG GTA				672
Lys Ile Phe Leu Gly Leu Ala Ile Thr Thr Met Cys Ile Asp Leu Val	210	215	220	
GGA GTA CAG TAT ATT CGA AAG ATT CAT TAT TTC GGA AGA AAA ATT CAA				720
Gly Val Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln	225	230	235	240
GAC GCT AGA TCT GCA TTG GCG GTT GTA GGA GGA AAG GTA GTC CTT GTA				768
Asp Ala Arg Ser Ala Leu Ala Val Val Gly Gly Lys Val Val Leu Val	245	250	255	
TCA GAA CTC TAC GCA AAT TTA ATG CAA AAG CGA GCT CGT AAC ATG TCC				816
Ser Glu Leu Tyr Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser	260	265	270	
CGA GAA GCT TTT ATA GTG GAG AAT CTC TAT GTT TCC AAA CAC ATC ATA				864
Arg Glu Ala Phe Ile Val Glu Asn Leu Tyr Val Ser Lys His Ile Ile	275	280	285	
CCA TTC ATA CCA ACT GAT ATC CGA TGT ATT CGA TAT ATT GAT CAA ACT				912
Pro Phe Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr Ile Asp Gln Thr	290	295	300	
GCC GAT GCT GCT ACC ATT TCC ACG TCA TCG TCT GCA ATT GAT ATG CAA				960
Ala Asp Ala Ala Thr Ile Ser Thr Ser Ser Ser Ala Ile Asp Met Gln	305	310	315	320
AGT TGT AGA TTT TGT CAT TCA AGA TAT TCT CTC AAT CGT GCA TTC AAA				1008
Ser Cys Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys	325	330	335	
TAG				1011

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 336 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ser	Asp	Gln	Leu	Phe	Val	Ala	Phe	Glu	Lys	Tyr	Phe	Leu	Thr	Ser
1				5					10				15		
Asn	Glu	Val	Lys	Lys	Asn	Ala	Ala	Thr	Glu	Thr	Trp	Thr	Phe	Ser	Ser
		20						25					30		
Ser	Ile	Phe	Phe	Ala	Val	Thr	Val	Thr	Thr	Ile	Gly	Tyr	Gly	Asn	
	35					40					45				

Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Cys Ile Leu Phe Ser
 50 55 60
 Leu Leu Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Asp Leu Ala Gly
 65 70 75 80
 Lys Phe Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys
 85 90 95
 Leu Lys Tyr Leu Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His
 100 105 110
 Val Cys Glu His Cys His Ser His Gly Met Gly His Asp Met Asn Ile
 115 120 125
 Glu Glu Lys Arg Ile Pro Ala Phe Leu Val Leu Ala Ile Leu Ile Val
 130 135 140
 Tyr Thr Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser
 145 150 155 160
 Phe Phe Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly
 165 170 175
 Phe Gly Asp Leu Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu
 180 185 190
 Leu Tyr Ile Ile Leu Gly Lys Phe Ser Met Lys Lys Lys Gln Lys Phe
 195 200 205
 Lys Ile Phe Leu Gly Leu Ala Ile Thr Thr Met Cys Ile Asp Leu Val
 210 215 220
 Gly Val Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln
 225 230 235 240
 Asp Ala Arg Ser Ala Leu Ala Val Val Gly Gly Lys Val Val Leu Val
 245 250 255
 Ser Glu Leu Tyr Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser
 260 265 270
 Arg Glu Ala Phe Ile Val Glu Asn Leu Tyr Val Ser Lys His Ile Ile
 275 280 285
 Pro Phe Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr Ile Asp Gln Thr
 290 295 300
 Ala Asp Ala Ala Thr Ile Ser Thr Ser Ser Ser Ala Ile Asp Met Gln
 305 310 315 320
 Ser Cys Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys
 325 330 335

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 51 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCCATTTTCT TTGCCGTAAC CGTCGTCACCT ACCATCGGAT ACGGTAATCC A

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 51 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCATTCTACT GGTCCCTTCAT TACAATGACT ACTGTCGGGT TTGGCGACTT G

51

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala	Phe	Leu	Phe	Ser	Ile	Glu	Thr	Gln	Thr	Thr	Ile	Gly	Tyr	Gly	Phe
1				5					10					15	

Arg	Cys	Val	Thr	Asp	Glu	Cys	Pro
				20			

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala	Phe	Leu	Phe	Ser	Leu	Glu	Thr	Gln	Val	Thr	Ile	Gly	Tyr	Gly	Phe
1				5					10					15	

Arg	Cys	Val	Thr	Glu	Gln	Cys	Ala
				20			

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala	Phe	Leu	Phe	Phe	Ile	Glu	Thr	Glu	Ala	Thr	Ile	Gly	Tyr	Gly	Tyr
1				5					10					15	

Arg	Tyr	Ile	Thr	Asp	His	Cys	Pro
				20			

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Phe Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn
1 5 10 15
Ile Ser Pro Thr Thr Phe Ala Gly
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Phe Trp Trp Ala Val Val Thr Met Thr Thr Val Gly Tyr Gly Asp
1 5 10 15
Met Thr Pro Val Gly Phe Trp Gly
20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Phe Trp Tyr Thr Ile Val Thr Met Thr Thr Leu Gly Tyr Gly Asp
1 5 10 15
Met Val Pro Glu Thr Ile Ala Gly
20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Phe Trp Trp Ala Gly Ile Thr Met Thr Thr Val Gly Tyr Gly Asp
1 5 10 15
Ile Cys Pro Thr Thr Ala Leu Gly
20

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Leu Trp Trp Ala Leu Val Thr Met Thr Thr Val Gly Tyr Gly Asp
 1 5 10 15
 Met Ala Pro Lys Thr Tyr Ile Gly
 20

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Leu Tyr Phe Thr Met Thr Cys Met Thr Ser Val Gly Phe Gly Asn
 1 5 10 15
 Val Ala Ala Glu Thr Asp Asn Glu
 20

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Cys Val Tyr Phe Leu Ile Val Thr Met Ser Thr Val Gly Tyr Gly Asp
 1 5 10 15
 Val Tyr Cys Glu Thr Val Leu Gly
 20

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ser Leu Tyr Thr Ser Tyr Val Thr Thr Thr Ile Gly Phe Gly Asp
 1 5 10 15

45

Tyr Val Pro Thr Phe Gly Ala Asn
20

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Phe Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn
1 5 10 15
Ile Ser Pro Thr Thr Phe Ala Gly
20

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Ile Phe Phe Ala Val Thr Val Val Thr Thr Ile Gly Tyr Gly Asn
1 5 10 15
Pro Val Pro Val Thr Asn Thr Gly
20

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ser Leu Tyr Thr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe Gly Asp
1 5 10 15
Tyr Val Pro Thr Phe Gly Ala Asn
20

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly Asp
 1 5 10 15
 Leu Met Pro Arg Arg Asp Gly Tyr
 20

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATAAAGCTTA AAAATGTCGC CGAATCGATG GAT

33

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGCTCTAGAC CTCCATCTGG AAGCCCATGT

30

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAAAAGCTTA AAATGGCACA CATCACG

27

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAACTCGAGT CATACCTGTG GACT

24

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAAAAGCTTA AAATGGTCGG GCAATTG

27

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AAAAGCATGC TCATCTGGAT GGGCA

25

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAAAAGCTTA AAATGGCCTC GGTCGCC

27

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTTTCTAGAC TACATCGTTG TCTT

24

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAAAAGCTTA AAATGAATCT GATCAAC

27

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAATCTAGAT TAGTCGAAAC TGAA

24

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAAAAGCTTA AAATGCCTGG CGGA

24

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AAATCTAGAG GCTACAGGAA GTCC

24

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGGGTACCA AAATGTCGGG GTGTGAT

27

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TTTTTCTAGA TCAAGAGTTA TCATC

25

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1529 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

Asx Asp Asp Ala His Asx Asp Asp Ala Asx His Ala Asx His Ala Asp
1           5           10           15
Asx Ala Asp Asx His Asp His Ala Ala His Ala Asx Asp Ala Asx His
20           25           30
Asx Ala Asx His Ala His Ala Asp Asp Asp Ala Ala His Ala His His
35           40           45
Ala Ala Asp Asx His Asp Asp His Ala Asx Asx Asp Ala Asp Asx His
50           55           60
Asx Asp Asp Ala His Asx Asx Ala Asx His Ala Asp His Ala Ala Asx
65           70           75           80
Asp Asp Asx Asx Asp Asx Asx Ala Asp His Asp His Asp Asp His Asp
85           90           95
Asp Ala Ala His His Asx Asp Asp Ala Ala Ala His Asp Asp His Ala
100          105          110
Ala His Ala Ala His Asx Ala Ala Asx Asx Asp Ala His Asx Asp Ala
115          120          125
Ala Asx Ala Asx Asx His Ala His Asp Asx Asx Asp His His Asp Ala
130          135          140
Asp Asx Ala Asp Asp Ala Ala Asp Asx Ala His His His Asx Asx Ala
145          150          155          160
Ala Asp Ala Asp Ala Asx Ala Ala Asp His Ala Asx Ala Ala His Ala
165          170          175
His His Asp His Asx His Ala Asx His Asp Asp Asx His Asx Asp His
180          185          190
Asx Ala His His Asx His His Asp His His Asp Asp Ala His His Asx
195          200          205
Asp Asp Ala Asp His His Asx His His Asx His Asp Asx Asx Ala His
210          215          220
Asp Asp Ala Ala His Ala His Asp His His Asx Ala His His Ala Asx
225          230          235          240
His Ala His Asx Asp Asx Asx Asx Asx His Asp Ala Asp His Ala His
245          250          255
His Ala His His Asp His Asp Ala Ala His His Ala His His Asp Asp
260          265          270
His His Asx Ala Ala Ala Asx Asx Asp Asp Ala His Asp Asp Asx Asp
275          280          285
His Asp Asp Ala Asp Ala Asx Ala His Asp Asp His Ala His His Asx
290          295          300
Asp Ala Ala Ala Asp Ala Ala His His Asx Ala His Asp Asp Asp Ala
305          310          315          320
His Asx His His His Asp Ala Asx Asp Ala His His Asp Asp Asx His
325          330          335
Asx Ala Asx Ala Ala Asx His His Asx Asx Ala Ala Ala Asx Asp Asx
340          345          350

```

Ala Ala Asp Asx Ala His His Ala Ala His Asp His His His His Asx
 355 360 365
 Ala Ala Asx Asx His Asp His His Asx Asx His Asx Ala His His Ala
 370 375 380
 His His Asp Asx His Asp Asp His Asx Asx Asx Asx His Asp Ala His
 385 390 395 400
 Asx His Ala Asx Asx Asp Asx Asp His Asx His His His Asp Asx His
 405 410 415
 Asx Asx Asp Asp His His His Asp Asx His His Asx Ala Ala Asx Ala
 420 425 430
 His Asx Asp His Asx Ala Ala Asx Asx His Asp Ala Asx Ala Ala His
 435 440 445
 Asx Ala His His Asx His Asx Ala His Asx Asx His Asx Asp His Asx
 450 455 460
 Ala His His Asp His His Asx His Asp Asp Ala Asp Asx Asx Asx Ala
 465 470 475 480
 Asx His Asp Ala Ala Asp Ala His His Asx Asx Ala His Asp His Asx
 485 490 495
 Asx His Asp Asp His His His His His Asx His Asp Asp Asp His Ala
 500 505 510

 Ala Asx His Asx His His Asp Asp Ala Ala His Asp Asp Asx Asp Ala
 515 520 525
 Asx His His Asx Ala Ala His Asx Ala Ala His His His His Ala Asp
 530 535 540
 Asx Ala Ala His Asp Asp Asp Asx His Ala His Ala His Asp Ala Ala
 545 550 555 560
 Ala Ala Asx His Asx Asp Asp His His His Ala His Asp Asp Ala Asp
 565 570 575
 His His Asp Asp His Asp Asp Asx Asp Ala His His His His Asx Asx
 580 585 590
 Asp Asx Ala Asx Ala Asx Asx His Ala Asx Ala His His Asp Asp Asx
 595 600 605
 Asp Asx His Asx His Asx His His Asp Ala His His Asp Asp Ala Ala
 610 615 620
 Ala Asx Ala Ala Asx Ala His His His Asp Asx Asp Asp Ala His His
 625 630 635 640
 Asp Asx His Asp Ala His Ala Ala Asx Asp Asp His His Asp His Asp
 645 650 655
 Ala Ala Ala Ala His Asx Asp Asp Ala Asp His Asp Ala Asx Asx His
 660 665 670
 Ala His His His His Asx His Asp Ala Ala His Asp Ala His Asp Ala
 675 680 685
 Asp Asx Asx His Ala Ala Ala Asx His His Asp His His Asp Asx Ala
 690 695 700
 Ala His Asx Asp His Asx His Ala His His His Asx Asp Asp Asx Ala
 705 710 715 720

His Ala His Asx Asp His His Asp Asp His Asp Ala His Asx Asx His
 725 730 735
 His Asx His Asp Asp His Asp His Asp His Asp Asx Ala Ala His His
 740 745 750
 Asp Asx Ala Asx His His His His Asx His His His Ala His Asx Ala
 755 760 765
 Asx Ala Ala Ala Asp Asx Ala Ala Asp Ala His His His Asx His Ala
 770 775 780
 Asx Asx Ala Asx His Ala His Asx Ala Asx Asx Ala His Asx Ala Ala
 785 790 795 800
 Ala Ala Ala Asp Asp Ala Ala His Asp Asp Ala Ala Ala His His Asx
 805 810 815
 Asp Asx Asp Ala Ala Ala Ala Asp Asp Asx Asp Asp Ala Ala Ala Asx
 820 825 830
 Asx Asp Ala Asx Ala Asp Ala Asx Asx Asp His Asx His Asx Asx Ala
 835 840 845
 His Asx Asx Ala His His Asx His His His Asp Asp Ala Asx Asx Ala
 850 855 860
 Asx Ala His His Asx Ala Asx Ala Ala Ala Asx His Asp His His Ala
 865 870 875 880
 His Asp Asp Asp Asx Ala Ala Asx His Asx His His Asx Ala Ala His
 885 890 895
 Asp His His His Asp Asp His His Asx His Asp Asx His His His Asp
 900 905 910
 Asx Asx Asp His His Ala Asx His Asx His Asx Ala Asx Ala Ala His
 915 920 925
 Asx His His Asx Asx Asx His Asp His His Ala His Asp Ala His Asp
 930 935 940
 Ala Asx Asx Asp His His Ala Asx Asx Ala Asx His Asx Asp His Asp
 945 950 955 960
 Asp Ala Asp Ala His His Asx Asx Asp Asp Asx His His Asx Asx His
 965 970 975
 Ala Ala Ala Asx Ala Ala Ala Ala His His Ala His Asp His Asx His
 980 985 990
 Asp Ala Ala Ala Ala Asx Asp Ala His Asp Ala Ala Ala His Asx His
 995 1000 1005
 Ala Asx Ala Asx His His His Asp Asx His Asx Ala Asx Ala Ala Asp
 1010 1015 1020
 His His His Asx Asx His Asx His His Asx His His Asx Ala Ala His
 1025 1030 1035 1040
 His His Asp His His Asx Asp Asx His Asp Asx Asp Ala His His Asp
 1045 1050 1055
 Asp Ala His Asx Asx Ala His Ala Asp His His Asp Asx His His Asx
 1060 1065 1070
 Asx Ala Ala Asp Ala His His Asx Ala Asx His Asp Asp Asx Asx Asp
 1075 1080 1085

Ala Asx Ala Asx Asx Asx Asx Asp His His Ala Asx Asx His Asx Ala
1090 1095 1100

Ala Ala His His His Asp Asx Asx Ala His Ala Ala His Asx His His
1105 1110 1115 1120

Asp Asx Asp His Asp Asx His Asx His His His His Asx Ala His His
1125 1130 1135

Asx Asx Ala His His Asx His His Asx His His Asx His His Asx His
1140 1145 1150

Asp Asx Ala Ala Asx His Ala His Asx Asp His Asp His Asx Asx Ala
1155 1160 1165

Asp Ala Asx Asp Asx Asp His Asp Asx His His Ala His Asx Asx His
1170 1175 1180

Asp His His His His Asx His His His Asp Ala Asp His Asx His Ala
1185 1190 1195 1200

Asx His Asp Ala Asx Ala His His His His His Asp His Asp Ala His
1205 1210 1215

His Asp Asp His Asp Asp Ala Ala His His Asp Asx Asx Ala His Asp
1220 1225 1230

His Asx His His His His His Asx Ala Asx Ala His Asp Asp Ala His
1235 1240 1245

Ala Asx Asx His Asx Ala Asp Asx Asp Asx His Asx His Asp Asp Asx
1250 1255 1260

Ala Ala His Asp Asp Asp Ala His Ala Asx Ala Asx His Asx Asx Ala
1265 1270 1275 1280

Ala Ala Asx Asp His Asx Asp His Asp Asx Asx Ala His Asx His Asx
1285 1290 1295

Ala Asx His Ala Asx His Asx Ala Ala Asp Ala His His His Asp Asx
1300 1305 1310

Asx Asp Asx His Asx Ala Asp Asx His His His Asx Asx Asp His His
1315 1320 1325

His Asp Asx Ala Asx His Asx His His Ala His Asp Asp His His Asp
1330 1335 1340

Asp Asx Asx His His Asx His Asx Ala Asx Asx Asp Asp His Asp Asp
1345 1350 1355 1360

Asx Asx His Asp His Asp Asp Asx Asx Asx Asp His His Asp His His
1365 1370 1375

Ala His His Asp Ala Asp Asx Ala Asx His His Asx Asp His Asp Asp
1380 1385 1390

Ala Asx Ala Ala Asp Asx Asx Ala Ala Asp His Ala His Asx His His
1395 1400 1405

Ala His Ala Ala Ala His Ala His His His Ala His Ala Asp Asx Ala
1410 1415 1420

His His Ala Asp Ala Asp His Ala His Ala Asx His His Asp His His
1425 1430 1435 1440

Ala His Ala His Asp His His Asp His His His His Ala His His
1445 1450 1455

53

Ala Ala Asp Asx His Asp His Asp Asp Ala Ala His Ala Ala Ala Ala
 1460 1465 1470

His Ala Ala His His Ala His His Ala Ala Ala Ala Ala Ala Ala Ala
 1475 1480 1485

Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Asp Asx Asp
 1490 1495 1500

Asx Asx Asp Asx His Asx Asp Ala Asp Asx Ala His His Asx Ala His
 1505 1510 1515 1520

Asp Asp Ala Asp Ala Asp Ala Ala Ala
 1525

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 479 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr Ile Ser Tyr
 1 5 10 15

~~Leu Met Phe Gly Ala Ala Ile Tyr Tyr His Ile Glu His Gly Glu Glu~~
 20 25 30

Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Gln Ile Ala Ile Asn Glu
 35 40 45

Tyr Leu Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr Gln Asp Glu
 50 55 60

Ile Leu Gln Arg Ile Ser Asp Tyr Cys Asp Lys Pro Val Thr Leu Pro
 65 70 75 80

Pro Thr Tyr Asp Asp Thr Pro Tyr Thr Trp Thr Phe Tyr His Ala Phe
 85 90 95

Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn Ile Ser
 100 105 110

Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr Ser Val Ile
 115 120 125

Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly Glu Tyr Phe
 130 135 140

Gly Arg Thr Phe Glu Ala Ile Tyr Arg Arg Tyr Lys Lys Tyr Lys Met
 145 150 155 160

Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu Ile Thr Thr
 165 170 175

Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu Val Leu Pro
 180 185 190

Cys Val Gly Val His Leu Leu Arg Glu Leu Gly Leu Ser Ser Ile Ser
 195 200 205

Leu Tyr Tyr Ser Tyr Val Thr Ile Thr Thr Ile Gly Phe Gly Asp Tyr
 210 215 220

Val Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Gly Gly Trp Phe
 225 230 235 240
 Val Val Tyr Gln Ile Phe Val Ile Val Trp Phe Ile Phe Ser Leu Gly
 245 250 255
 Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu Gln Ser Lys
 260 265 270
 Lys Leu Ala Tyr Leu Glu Gln Gln Leu Ser Ser Asn Leu Lys Ala Thr
 275 280 285
 Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val Gly Tyr Leu Arg
 290 295 300
 Arg Met Leu Asn Glu Leu Tyr Ile Leu Lys Val Lys Pro Val Tyr Thr
 305 310 315 320
 Asp Val Asp Ile Ala Tyr Thr Leu Pro Arg Ser Asn Ser Pro Leu Ser
 325 330 335
 Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser Arg Lys Arg Ala Phe
 340 345 350
 Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg Glu Ala Gly Met Val
 355 360 365
 His Ala Asn Ser Asp Thr Asp Leu Thr Lys Leu Asp Arg Glu Lys Thr
 370 375 380
 Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr Asp Leu Leu Ala Lys
 385 390 395 400
 Val Val Asn Ala Leu Ala Thr Val Lys Pro Pro Pro Ala Leu Gln Glu
 405 410 415
 Asp Ala Ala Leu Tyr Gly Gly Tyr His Gly Phe Ser Asp Ser Gln Ile
 420 425 430
 Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn Glu Phe Thr Ser Pro
 435 440 445
 Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe Asn Leu Glu Ala Pro
 450 455 460
 Arg Trp Gln Ser Glu Arg Pro Leu Arg Ser Ser His Asn Glu Trp
 465 470 475

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 335 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Ser Asp Gln Leu Phe Val Ala Phe Glu Lys Tyr Phe Leu Thr Ser
 1 5 10 15
 Asn Glu Val Lys Lys Asn Ala Ala Thr Glu Thr Trp Thr Phe Ser Ser
 20 25 30
 Ser Ile Phe Phe Ala Val Thr Val Val Thr Thr Ile Gly Tyr Gly Asn
 35 40 45

Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Ile Leu Phe Ser Leu
 50 55 60
 Ile Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Leu Ala Gly Lys Phe
 65 70 75 80
 Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys Leu Lys
 85 90 95
 Tyr Leu Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His Val Cys
 100 105 110
 Glu His Cys His Ser His Gly Met Gly His Asp Met Asn Ile Glu Glu
 115 120 125
 Lys Arg Ile Pro Ala Phe Leu Val Leu Ala Ile Leu Ile Val Tyr Thr
 130 135 140
 Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser Phe Phe
 145 150 155 160
 Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly
 165 170 175
 Asp Leu Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu Leu Tyr
 180 185 190
 Ile Ile Leu Gly Lys Phe Ser Met Lys Lys Lys Gln Lys Phe Lys Ile
 195 200 205
 Phe Leu Gly Leu Ala Ile Thr Thr Met Cys Ile Asp Leu Val Gly Val
 210 215 220
 Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln Asp Ala
 225 230 235 240
 Arg Ser Ala Leu Ala Val Val Gly Gly Lys Val Val Leu Val Ser Glu
 245 250 255
 Leu Tyr Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser Arg Glu
 260 265 270
 Ala Phe Ile Val Glu Asn Leu Tyr Val Ser Lys His Ile Ile Pro Phe
 275 280 285
 Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr Ile Asp Gln Thr Ala Asp
 290 295 300
 Ala Ala Thr Ile Ser Thr Ser Ser Ser Ala Ile Asp Met Gln Ser Cys
 305 310 315 320
 Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys Xaa
 325 330 335

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What is claimed is:

1. A potassium channel comprising four hydrophobic domains capable of forming transmembrane helices, wherein
 - (i) a first pore-forming domain is interposed between a first and a second transmembrane helix; and
 - (ii) a second pore-forming domain is interposed between a third and a fourth transmembrane helix.
2. The potassium channel of claim 1 wherein each pore-forming domain comprises a potassium selective peptide motif.
3. The potassium channel of claim 2 wherein the peptide motif is selected from the group consisting of a Y/F-G dipeptide motif and a G-Y/F-G tripeptide motif.
4. The potassium channel of claim 3 wherein at least one pore-forming domain is positioned proximal to an exterior portion of a cell membrane.
5. The potassium channel of claim 4 further comprising an amino-terminal glycosylation site.
6. The potassium channel of claim 5 wherein said glycosylation site is asparagine-linked.
7. The potassium channel of claim 6 characterized in that it belongs to a class of invertebrates.
8. The potassium channel of claim 7 characterized in that

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it is insect-derived.

9. The potassium channel of claim 7 characterized in that it is nematode-derived.

10. An isolated nucleotide sequence capable of encoding DmORF-1.

11. The isolated nucleotide sequence of Claim 10 comprising the nucleotide sequence depicted in Seq. I.D. No. 1.

12. An isolated nucleotide sequence capable of encoding CORK.

13. The isolated nucleotide sequence of Claim 12 encoding for the protein depicted in Sequence I.D. No. 36.

14. An expression vector capable of expressing a heterologous potassium channel in a cell membrane of a yeast cell comprising the nucleotide sequence of Claim 10.

15. An expression vector capable of expressing a heterologous, potassium channel in a cell membrane of a yeast cell comprising the nucleotide sequence of Claim 11.

16. An expression vector capable of expressing a heterologous potassium channel in a cell membrane of a yeast cell comprising the nucleotide sequence of Claim 12.

17. An expression vector capable of expressing a heterologous potassium channel in a cell membrane of a yeast cell wherein the potassium channel comprises the amino acid sequence of Claim 13.

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18. A transformed yeast cell comprising the nucleotide sequences of Claims 10, 11, 12 or 13.

19. A transformed yeast cell comprising the expression vector of claims 14, 15, 16 or 17.

20. A method of assaying substances to determine effects on cell growth, the method comprising the steps of:

- a. preparing cultures of yeast cells in liquid medium lacking uracil, the liquid medium consisting of a concentration of KCl adequate to support growth of potassium-dependent mutant strains;
- b. plating the yeast cells in uracil-free agar medium, the agar medium consisting of sufficient KCl to selectively support growth of potassium-dependent mutant strains containing a heterologous potassium channel of claim 1;
- c. applying substances to the agar plate;
- d. incubating the agar plate to permit growth; and
- e. identifying zones of growth around the substances, wherein the level of growth indicates whether or not activity of the heterologous potassium channel has been modulated as compared to control.

21. The yeast cell of Claim 20 further comprising a nucleotide sequence encoding RAK, or a nucleotide sequence of Claim 10, 11, 12 or 13.

22. The method of claim 20, wherein said effect on cell

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growth is modulated by activation of the potassium channel.

23. The method of claim 20, wherein said effect on cell growth is modulated by inhibition of said potassium channel.

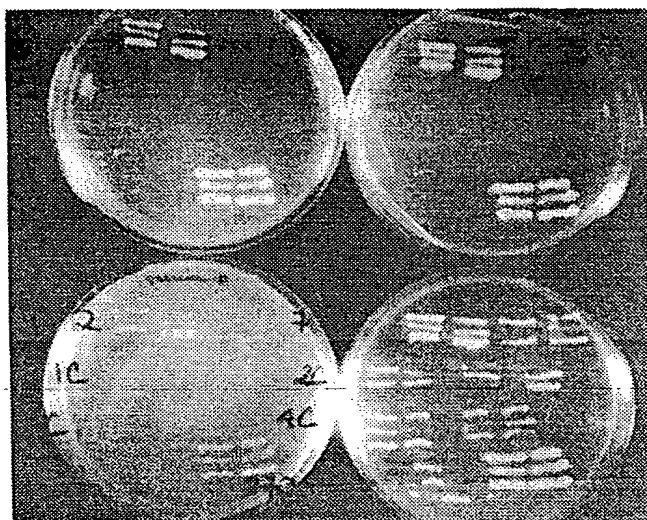
24. A method of selectively inhibiting insect pests by applying to such insect pests a substance capable of inhibiting a potassium channel substantially homologous to that encoded by the nucleotide sequence of claim 10.

25. A method of selectively inhibiting nematode pests by applying to such pests a substance capable of inhibiting a potassium channel substantially homologous to that encoded by the nucleotide sequence of claim 12.

26. A method of modulating the activity of a potassium channel positioned in a cellular membrane and comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 36, by contacting said cellular membrane with a substance, in an amount and for a period of time sufficient to inhibit the ability of potassium ions to pass through said channel.

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SC galactose, 100 mM KCl

SC glucose, 0mM KCl

SC galactose, 0 mM KCl

SC glucose, 100 mM KCl

FIG. 1

growth of *Y. lipolytica* on different carbon sources

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5' ...ACGCGATCGCGGAGTGATATTTTATTTTATTTAGCTCAGTCTCAGTGTTCGGATTCTCTTTAAAGAAAAAATAATAGTCAA
AACTACAAACACAGCGAAAGCGGAAAGCAACGGTCTCTCGAGTGTATTTTTCACAAATTTTATGATCGTAGTCGACAAATCCGTCGAGC

Met Ser Pro Asn Arg Trp Ile Leu Leu Ile Phe Tyr Ile Ser Tyr Leu Met Phe Gly Ala Ala Ile Tyr Tyr
ATG TCG CCG AAT CGA TGG ATC CTG CTG ATC TTC TAC ATA TCC TAC CTG ATG TTC GGG CGG GCA ATC TAT TAC
His Ile Glu His Gly Glu Glu Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Gln Ile Ala Ile Asn Glu Tyr Leu
CAT ATT GAG CAC GGC GAG AAG ATA TCG CGC GCC GAA CAG CGC AAG GCG CAA ATT GCA ATC AAC GAA TAT CTG
Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr Gln Asp Glu Ile Leu Gln Arg Ile Ser Asp Tyr Cys Asp Lys
CTG GAG GAG CTG GGC GAC AAG AAT ACG ACC ACA CAG GAT GAG ATT CTT CAA CCG ATC TCG GAT TAC TGT GAC AAA
Pro Val Thr Leu Pro Pro Thr Tyr Asp Asp Thr Thr Thr Pro Thr Thr Thr Phe Tyr His Ala Phe Phe Ala Phe
CCG GTT ACA TTG CCG CCG ACA TAT GAT GAT ACG CCC TAC ACG TGG ACC TTC TAC CAT GCC TTC TTC GCG TTC
Thr Val Cys Ser Thr Val Gly Tyr Gly Asn Ile Ser Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr
ACC GTT TGC TCC ACG GTG GGA TAT GGG AAT ATA TCG CCA ACC ACC TTC GCC GGA CGG ATG ATC ATG ATC GCG TAT
Ser Val Ile Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly Glu Tyr Phe Gly Arg Thr Phe Glu Ala
TCG GTG ATT GGC ATC CCC GTC AAT GGT ATC CTC TTT GCC GGC CTC GGC GAA TAC TTT GGA CGT ACG TTT GAA GCG
Ile Tyr Arg Arg Tyr Lys Tyr Lys Met Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu Ile Thr
ATC TAC AGA CGC TAC AAA AAG TAC AAG ATG TCC ACG GAT ATG CAC TAT GTC CCG CCG CAG CTG GGA TTG ATC ACC
Thr Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu Val Leu Pro Cys Val Gly Val His Leu Leu Arg
ACG GTG GTG ATT GCC CTG ATT CCG GGA ATA GCT CTC TTC CTG GTG CTG CCC TGC GTG GGT GTT CAC CTA CTT CGA
Glu Leu Gly Leu Ser Ile Ser Leu Tyr Tyr Ser Tyr Val Thr Thr Thr Ile Gly Phe Gly Asp Tyr Val
GAA CTG GGC CTA TCT TCC ATC TCG CTG TAC TAC AGC TAT GTG ACC ACC ACA ACA ATT GGA TTC GGT GAC TAT GTG
Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Phe Gly Gly Trp Phe Val Val Tyr Gln Ile Phe Val Ile Val Trp
CCC ACA TTT GGA GCC AAC CAG CCC AAG GAG TTC GGC GGC TGG TTC GTG GTC TAT CAG ATC TTT GTG ATC GTG TGG
Phe Ile Phe Ser Leu Gly Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu Gln Ser Lys Lys Leu Ala
TTC ATC TTC TCG CTG GGA TAT CTT GTG ATG ATC ATG ACA TTT ATC ACT CGG GGC CTC CAG AGC AAG AAG CTG GCA
Tyr Leu Glu Gln Leu Ser Ser Ser Asn Leu Lys Ala Thr Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val
TAC CTG GAG CAG CAG TTG TCC TCC AAC CTG AAG GCC ACA CAG AAT CCG ATC TCG TCT TGT GGC GTC ACC AAG GAT GTG
Gly Tyr Leu Arg Arg Met Leu Asn Glu Leu Tyr Tyr Ile Leu Lys Val Lys Pro Val Tyr Thr Asp Val Asp Ile Ala
GGC TAC CTC CGG CGA ATG CTC AAC GAG CTG TAC ATC CTC AAA GTG AAG CCT GTG TAC ACC GAT GTA GAT ATC GCC

FIG. 2A

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330	Tyr	Thr	Leu	Pro	Arg	Ser	Asn	Ser	Cys	Pro	Asp	Leu	Ser	Met	Tyr	Arg	Val	Glu	Pro	Ala	Pro	Ile	Pro	Ser	Arg	350	
	TAC	ACA	CTG	CCA	CGT	TCC	AAT	TCG	TGT	CCG	GAT	CTG	AGC	ATG	TAC	CGC	GTG	GAG	CCG	GCT	CCC	ATT	CCC	AGC	CGG	1050	
										360										370							
	Lys	Arg	Ala	Phe	Ser	Val	Cys	Ala	Asp	Met	Val	Gly	Ala	Gln	Arg	Glu	Ala	Gly	Met	Val	His	Ala	Asn	Ser	Asp	1125	
	AAG	AGG	GCA	TTC	TCC	GTG	TGC	GCC	GAC	ATG	GTT	GGC	GCC	CAA	AGG	GAG	GCG	GCG	ATG	GTA	CAC	GCC	AAT	TCC	GAT	400	
					380										390												
	Thr	Asp	Leu	Thr	Lys	Leu	Asp	Arg	Glu	Lys	Thr	Phe	Thr	Glu	Thr	Ala	Ala	Tyr	His	Gln	Thr	Thr	Asp	Leu	Leu	1200	
	ACG	GAT	CTA	ACC	AAA	CTG	GAT	CGC	GAG	AAG	ACA	TTC	GAG	ACG	GCG	GAG	GCG	TAC	CAC	CAG	ACC	ACC	GAT	TTG	CTG		
										410										420							
	Ala	Lys	Val	Val	Asn	Ala	Leu	Ala	Thr	Val	Lys	Pro	Pro	Pro	Ala	Glu	Gln	Glu	Asp	Ala	Ala	Leu	Tyr	Gly	Gly	1275	
	GCC	AAG	GTG	GTC	AAC	GCA	CTG	GCC	ACG	GTG	AAG	CCA	CCG	CCG	GCG	GAA	CAG	GAA	GAT	GCG	GCT	CTC	TAT	GGT	GGC	450	
					430										440												
	Tyr	His	Gly	Phe	Ser	Asp	Ser	Gln	Ile	Leu	Ala	Ser	Phe	Thr	Tyr	Thr	Val	Asn	Glu	Phe	Thr	Thr	Ser	Pro	Pro	1350	
	TAT	CAT	GGC	TTC	TCC	GAC	TCC	CAG	ATC	CTG	GCC	AGC	AGC	GAA	TGG	TCG	TTC	TCG	ACG	GTC	AAC	GAG	TTC	ACA	TCA	CCG	
									460											470							
	Arg	Arg	Pro	Arg	Ala	Arg	Ala	Cys	Ser	Asp	Phe	Asn	Leu	Glu	Ala	Pro	Arg	Trp	Gln	Ser	Glu	Arg	Pro	Leu	Arg	1425	
	CGA	CGT	CCA	AGA	GCA	CGT	GCC	TGC	TCC	GAT	TTC	AAT	CTG	GAG	GCA	CCT	CGC	TGG	CAG	AGC	GAG	AGG	CCA	CTG	CGT	500	
					480										490												
	Ser	Ser	His	Asn	Glu	Trp	Thr	Thr	Trp	Ser	Gly	Asp	Asn	Gln	Gln	Ile	Gln	Glu	Ala	Phe	Asn	Gln	Arg	Tyr	Lys	Gly	1500
	TCG	AGC	CAC	AAC	GAA	TGG	ACA	TGG	AGC	GGC	GAC	AAC	CAG	CAG	ATC	ATC	CAG	GAG	GCA	TTC	AAC	CAG	CGC	TAC	AAG	GGA	
										510										520							
	Gln	Gln	Arg	Ala	Asn	Ser	Gly	Ala	Ala	Asn	Ser	Thr	Met	Val	His	Leu	Glu	Pro	Asp	Ala	Leu	Glu	Gln	Leu	Arg	1575	
	CAG	CAG	CGT	GCC	AAC	GGA	GCA	GCC	AAC	TCG	ACC	ATG	ATG	GTC	CAT	CTG	GAG	CCG	GAT	GCT	TTG	GAG	GAG	CAG	CTG	AGA	550
					530										540												
	Asn	Asn	His	Arg	Val	Pro	Val	Ala	Ser	Arg	Ser	Ser	Ser	Pro	Cys	Arg	Met	Val	Cys	Asp	Val	Cys	Phe	Pro	Ser	Arg	1650
	AAC	AAT	CAC	CGG	GTG	CCG	GTC	CGG	TCA	AGA	AGT	TCT	CCA	T													

FIG. 2B

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10
 Met Ser Asp Gln Leu Phe Val Ala Phe Glu Lys Tyr Phe Leu Thr Ser Asn Glu Val Lys 20
 ATG TCC GAT CAG CTG TTT GTC GCA TTT GAG AAG TAT TTC TTG ACG AGT AAC GAG GTC AAG 60
 30
 Lys Asn Ala Ala thr Glu Thr Trp Thr Phe Ser Ser Ser Ile Phe Ala Val Thr Val 40
 AAG AAT GCA GCA ACG GAG ACA TGG ACA TTT TCA TCG TCC ATT TTC TTT GCC GTA ACC GTC 120
 H5-1 50
 Val Thr Thr Ile Gly Tyr Gly Asn Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Cys 60
 GTC ACT ACC ATC GGA TAC GGT AAT CCA GGT CCA GTG ACA AAC ATT GGA CGG ATA TGG TGT 180
 M2 70
 Ile Leu Phe Ser Leu Leu Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Asp Leu Ala Gly 80
 ATA TTG TTC TCC TTG CTT GGA ATA CCT CTA ACA CTG GTT ACC ATC GCT GAC TTG GCA GGT 240
 90
 Lys Phe Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys Leu Lys Tyr Leu 100
 AAA TTC CTA TCT GAA CAT CTT GTT TGG TTG TAT GGA AAC TAT TTG AAA TTA AAA TAT CTC 300
 110
 Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His Val Cys Glu His Cys His Ser His 120
 ATA TTG TCA CGA CAT CGA AAA GAA CGG AGA GAG CAC GGT TGT GAG CAC TGT CAC AGT CAT 360
 130
 Gly Met Gly His Asp Met Asn Ile Glu Glu Lys Arg Ile Pro Ala Phe Leu Val Leu Ala 140
 GGA ATG GGG CAT GAT ATG AAT ATC GAG GAG AAA AGA ATT CCT GCA TTC CTG GTA TTA GCT 420
 150
 M3
 Ile Leu Ile Val Tyr Thr Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser 160
 ATT CTG ATA GTA TAT ACA GCG TTT GGC GGT GTC CTA ATG TCA AAA TTA GAG CCG TGG TCT 480

FIG. 3A

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H5-2

170 180

Phe Phe Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly Asp Leu
 TTC TTC ACT TCA TTC TAC TGG TCC TTC ATT ACA ATG ACT ACT ACT GTC GGG TTT GGC GAC TTG 540
 190 200

Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu Leu Tyr Ile Ile Leu Gly Lys Phe
 ATG CCC AGA AGG GAC GAC TAC ATG TAT ATA TTT CTC TAT ATC ATT TTA GGT AAA TTT 600
 210 220

Ser Met Lys Lys Lys Gln Lys Phe Lys Ile Phe Leu Gly Leu Ala Ile Thr Thr Met Cys
 TCA ATG AAA AAA AAA CAA AAA TTC AAA ATA TTT TTA GGT CTT GCA ATA ACT ACA ATG TGC 660
 230 240

Ile Asp Leu Val Gly Val Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln
 ATT GAT TTG GTA GGA GTA CAG TAT ATT CGA AAG ATT CAT TAT TTC GGA AGA AAA ATT CAA 720
 250 260

Asp Ala Arg Ser Ala Leu Ala Val Val Gly Lys Val Val Leu Val Ser Glu Leu Tyr
 GAC GCT AGA TCT GCA TTG GCG GTT GTA GGA GGA AAG GTA GTC CTT GTA TCA GAA CTC TAC 780
 270 280

Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser Arg Glu Ala Phe Ile Val Glu Asn
 GCA AAT TTA ATG CAA AAG CGA GCT CGT AAC ATG TCC CGA GAA GCT TTT ATA GTG GAG AAT 840
 290 300

Leu Tyr Val Ser Lys His Ile Ile Pro Phe Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr
 CTC TAT GTT TCC AAA CAC ATC ATA CCA TTC ATA CCA ACT GAT ATC CGA TGT ATT CGA TAT 900
 310 320

Ile Asp Gln Thr Ala Asp Ala Ala Thr Ile Ser Thr Ser Ser Ala Ile Asp Met Gln
 ATT GAT CAA ACT GCC GAT GCT GCT ACC ATT TCC ACG TCA TCG TCT GCA ATT GAT ATG CAA 960
 330 336

Ser Cys Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys
 AGT TGT AGA TTT TGT CAT TCA AGA TAT TCT CTC AAT CGT GCA TTC AAA TAG 1011

FIG. 3B

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Ce orf1	-----	-----	-----	-----	-----		
Dm orf1	MSPNRWILL	LL IFYISYLMFG	AAIYYHIEHG	EEKISRAEQR	KAQIAINEYL	50	
Consensus	50	
Ce orf1	-----	--MSDQLFVA	FEKYFLTSNE	VKKNAATE	TW TFSSSI	FFAV 38	
Dm orf1	LEELGDKNTT	TQDEILQRIS	DYCDKPVTL	P	TYDDTPY	TFYHAF	FFAF 100
ConsensusTW TF.....FFA.....	100	
Ce orf1	TVVTITGYGN	PVPMINIGRI	WCILFSLGI	PLTLVTIAOL	AGKFLSEHLV	88	
Dm orf1	TVCSITGYGN	ISPTIFAGRM	IMIAYSVIGI	PVNGILFAGL	-----	140	
Consensus	TV..T..GYGN	..P..T..GR	..I..S..GI	P.....A..L	150	
Ce orf1	WLYGNYLKLK	YLILSRHRKE	RREHVCEHCH	SHGMGHDMNI	EEKRIPAF	FLV 138	
Dm orf1	--GEYFGRT	FEAIYRRYKK	YKMSTDMHYV	PPQLGLITTV	VIALIPGIAL	187	
Consensus	...G.Y....	...R..K..	...H..	...C....	...IP....	200	
Ce orf1	LAILIVYTAF	GGVLMASKLEP	WSFFTSTFYWS	FITIMTTMGFG	DLMPRRDGYM	188	
Dm orf1	FLVLPCVGVH	LLRELGLSS-	-----ISLYMS	YVITITTTGFG	DYVET-EGAN	231	
Consensus	...L.....S.Y.S.	...T.TT.GFG	D...P...G..	250	
Ce orf1	YIILLYIILG	KFSMKKKQKF	KIFLGLAITT	MCIDLWGVQY	IRKIHVFGRK	238	
Dm orf1	QPKEFGGWV	FV VYQIFVIVWF	IFSLGYLVMI	MTFITRGLOS	KKLAYLEQQL	281	
ConsensusF.....	...LG.....	...M.....	...G.C....	300	
Ce orf1	IQDARSALAV	VGGKVLVSE	LYANLMQKRA	RNMSREAFIV	ENLYVSKHII	288	
Dm orf1	SSNLKATQNR	IWSGVTKDVG	YLRRMLNELY	ILKVKPVYTD	VDIAYTLPRS	331	
ConsensusV.....	350	
Ce orf1	PFITDIRCI	-RYIDQTADA	ATISTSSSAI	DMOSCRFCHS	RYSLNRAFKX	337	
Dm orf1	NSCPOLSMYR	VEPAPIPSRK	RAFSVCADMV	GAOREAGMVH	ANSOTDLTKL	381	
Consensus	...E.....S.....	...Q.....	...S.....K....	400	
Ce orf1	-----	-----	-----	-----	-----	337	
Dm orf1	DREKTFETAE	AYHQTTDLLA	KVVNALATVK	PPPAEQEDAA	LYGGYHGFSD	431	
Consensus	450	
Ce orf1	-----	-----	-----	-----	-----	337	
Dm orf1	SQILASEWSF	STVNEFTSPR	RPRARACSDF	NLEAPRWQSE	RPLRSSHNEW	481	
Consensus	500	

FIG. 4

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FIG. 5A

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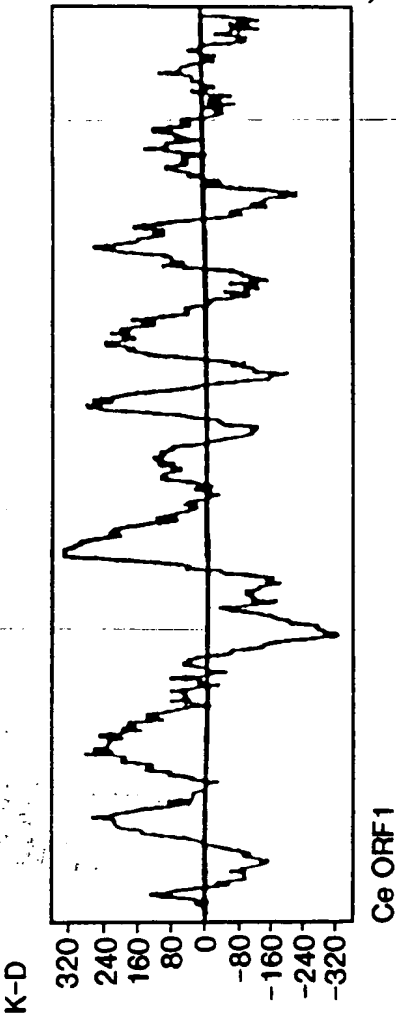
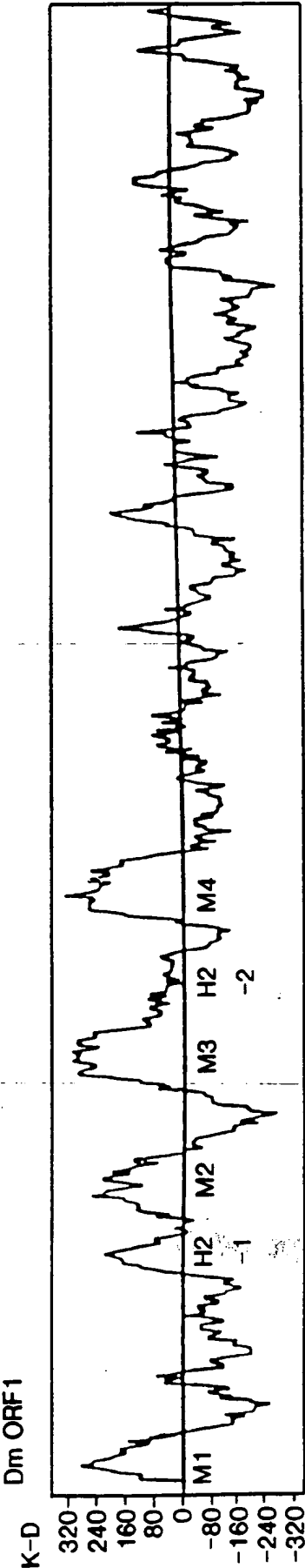
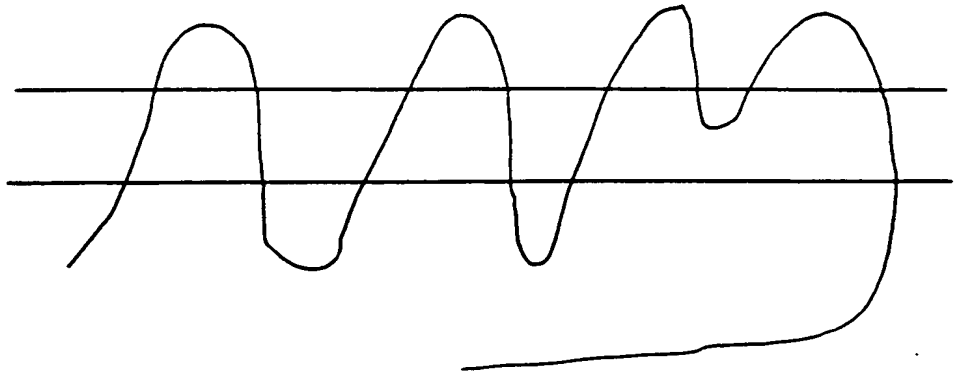


FIG. 5B

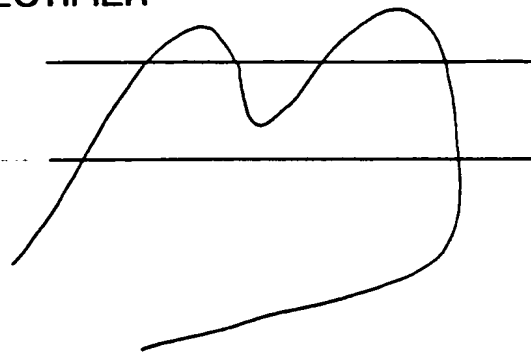
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1) SHAKER



2) INWARD RECTIFIER



3) ORF1

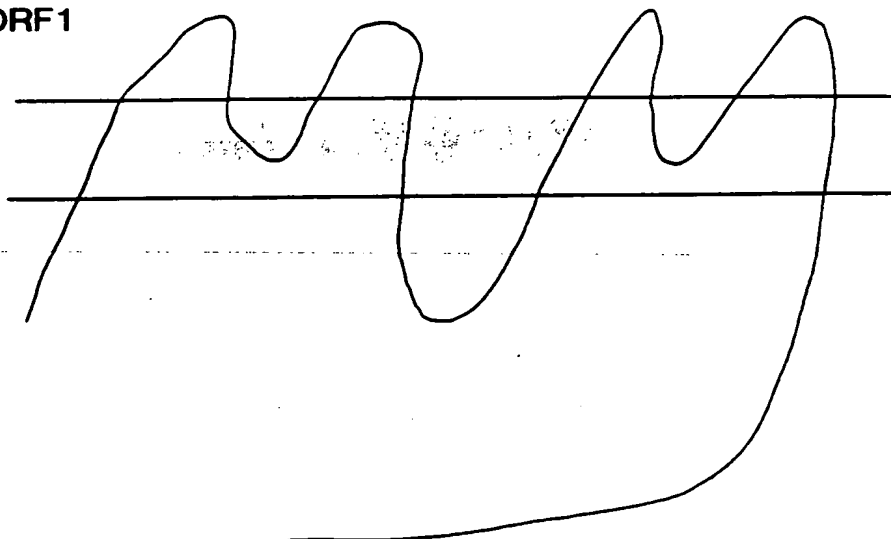


FIG. 6

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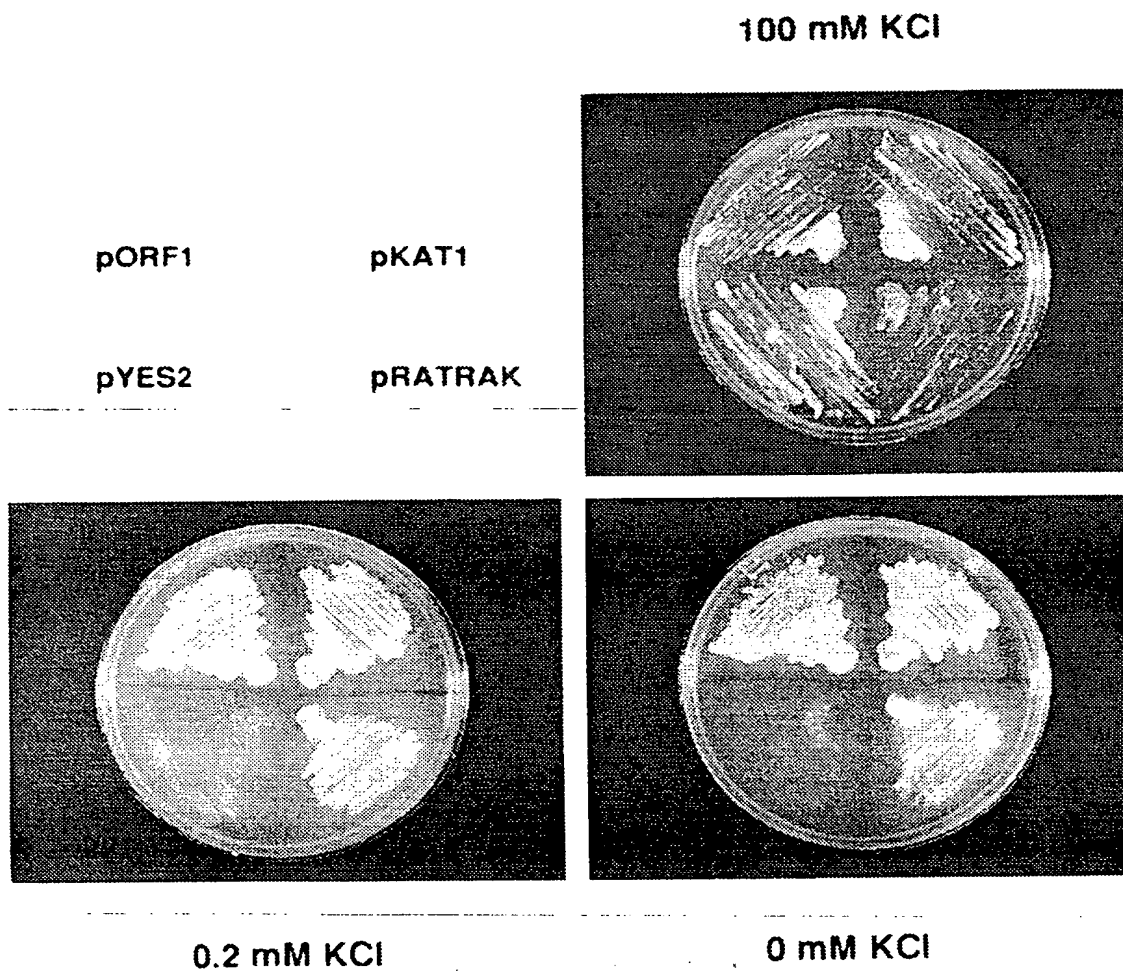


FIG. 7

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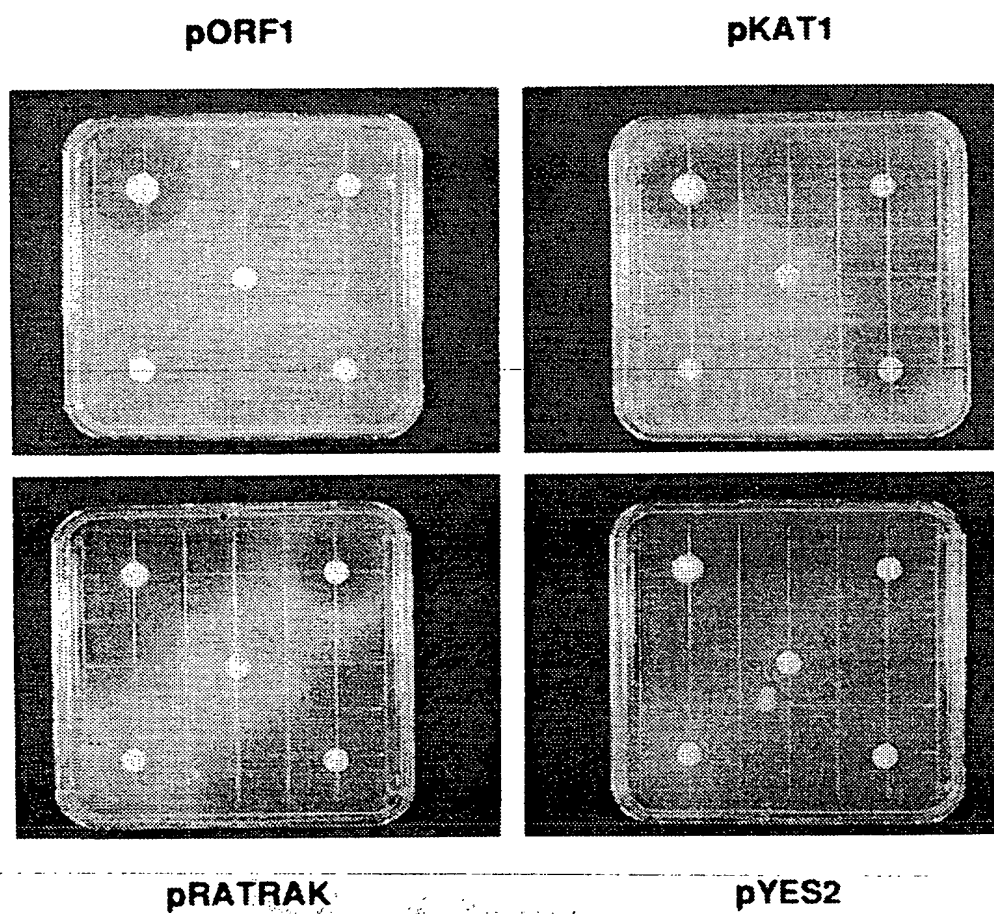


FIG. 8

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Met Val Ile Ile Asn Arg Ser Asn Thr Tyr Ala Val Glu Gln Glu Ala Phe Pro Arg Asp Lys Tyr Asn Ile Val
 ATG GTA ATA ATC AAC CGA TCG AAC ACC TAT TAT GCC GTT GAG CAG GAA GCA TTT CCA AGA GAC AAG TAC AAT ATT GTC
 10 20 75
 Tyr Trp Leu Val Ile Leu Val Gly Phe Gly Val Leu Leu Pro Trp Asn Met Phe Ile Thr Ile Ala Pro Glu Tyr
 TAC TGG CTC GTC ATT CTT CTT GGA TTC GGA GGT CTT CTT CTG CCA TGG AAT ATG TTC ATT ACT ATC GCC CCT GAG TAT
 30 40 50
 Tyr Val Asn Tyr Trp Phe Lys Pro Asp Gly Val Glu Thr Trp Tyr Ser Lys Glu Phe Met Gly Ser Leu Thr Ile
 TAT GTG AAT TAT TGG TTC AAA CCG GAT GGC GAG ACA TGG TAT TCG AAA GAA TTC ATG GGA TCT TTG ACG ATT
 60 70 225
 Gly Ser Gln Leu Pro Asn Ala Ser Ile Asn Val Phe Asn Leu Phe Ile Ala Gly Pro Leu Ile Tyr Arg
 GGC TCA CAA CTT CCA AAC GCA AGC ATT AAT GGT TTC AAC CTG TTC CTC ATT ATT GCT GGT CCC CTG ATC TAC CGC
 80 90 100
 Val Phe Ala Pro Val Cys Phe Asn Ile Val Asn Leu Thr Ile Ile Leu Val Ile Val Leu Glu Pro Thr
 GTC TTT GCT CCG GTT TGC TTC AAC ATC GTC AAC CTG ACA ATC ATT CTC ATC CTC GTC ATT GTT CTG GAG CCC ACT
 110 120 375
 Glu Asp Ser Met Ser Trp Phe Phe Trp Val Thr Leu Gly Met Ala Thr Ser Ile Asn Phe Ser Asn Gly Leu Tyr
 GAA GAT TCC ATG TCC TGG TTT TTC TGG GTA ACT CTT GGA ATG GCG ACT TCA ATC AAT TTT AGC AAT GGG CTA TAT
 130 140 150
 Glu Asn Ser Val Tyr Gly Val Gly Asp Phe Pro His Thr Tyr Ile Gly Ala Leu Leu Ile Gly Asn Asn Ile
 GAA AAC TCG GTT TAT GGA GGT GGT GGC GAT TTT CCG CAC ACC TAC ATT GGC GCT CTC TTG ATT GGA AAC AAC ATT
 160 170 525
 Cys Gly Leu Leu Ile Thr Val Val Lys Ile Gly Val Thr Tyr Phe Leu Asn Asp Glu Pro Lys Leu Val Ala Ile
 TGC GGA TTG CTG ATA ACG GTT GTG AAA ATC GGA GTG ACC TAT TTT CTG AAT GAT GAG CCT AAA CTT GTT GCA ATC
 180 190 200
 Val Tyr Phe Gly Ile Ser Leu Val Ile Leu Leu Val Cys Ala Ile Ala Leu Phe Phe Ile Thr Lys Gln Asp Phe
 GTC TAT TTC GGC ATA TCG TTG GTG ATC CTT CTG GTG TGT GCA ATT GCA CTT TTC TTT ATC ACA AAG CAA GAT TTC
 210 220 675

FIG. 9A

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230 Tyr His Tyr His His Gln Lys Gly Met Glu Ile Arg Glu Lys Ala Glu Thr Asp Arg Pro Ser Pro Ser Ile Leu 250
 TAC CAC TAT CAC CAC CAT CAA AAA GGA ATG GAA ATT CGC GAA AAG CCG TCT CCA TCC ATT CTT 750
 260 Trp Thr Thr Phe Thr Asn Cys Tyr Gly Gln Leu Phe Asn Val Trp Phe Cys Phe Ala Val Thr Leu Thr Ile Phe
 TGG ACC ACA TTC ACA AAC ACC GTT ACC ACT Thr Arg Gly Asp Ser Gly Phe Leu Phe Asn Val Trp TGG TTC TGC TTT GCC GTT ACT CTC ACA ATC TTC 825
 280 Pro Val Met Met Thr Val Thr Thr Arg Gly Asp Ser Gly Phe Leu Asn Lys Ile Met Ser Glu Asn Asp Glu Ile 300
 CCT GTT ATG ATG ACC GTT ACC GTT ACC ACT Thr Arg Gly Asp Ser Gly Phe Leu Asn Lys Ile Met Ser Glu Asn Asp Glu Ile
 310 Tyr Thr Leu Leu Thr Ser Phe Leu Val Phe Asn Leu Phe Ala Ala Ile Gly Ser Ile Val Ala Ser Lys Ile His 320
 TAC ACT TTG CTC ACA AGT TTC CTC GTC TTC AAT TTG TTC GCT GCG GCT CTA AAC AAA ATT ATG TCT TCT GAA AAC GAT GAA ATC 900
 330 Trp Pro Thr Pro Arg Tyr Leu Lys Phe Ala Ile Ile Leu Arg Ala Leu Phe Ile Pro Phe Phe Phe Cys Asn 350
 TGG CCG ACA CCC CGT TAC CTC AAA TTT GCC ATA ATC TTG CGT GCT CTT CTT TTC ATT CCA TTC TTC TTC TTC TGC AAC 1050
 360 Tyr Arg Val Gln Thr Arg Ala Tyr Pro Val Phe Phe Glu Ser Thr Asp Ile Phe Val Ile Gly Gly Ile Ala Met 370
 TAT CGT GTC CAG ACG CGT GCT TAT CCT GTT TTC TTT GAG TCT ACT ACT GAC ATT TTT GTG ATT GGT GGA ATT GCC ATG 1125
 380 Ser Phe Ser His Gly Tyr Leu Ser Ala Leu Ala Met Gly Tyr Thr Thr Pro Asn Val Val Pro Ser His Tyr Ser Arg 400
 TCT TTT TCA CAT GGA TAC CTC AGC GCT GCT CTG GCA ATG GGA TAC ACT CCA AAC GTC GTG CCA TCT CAC TAC TCA AGA 1200
 410 Phe Ala Ala Gln Leu Ser Val Cys Thr Leu Met Val Gly Gly Leu Thr Gly Gly Leu Trp Pro Val Val Ile Glu 420
 TTT GCC GCT CAG CTT TCC GTT TGC ACT CTT ATG GGT GGC CTT CTC ACC GGT GGC CTG TGG CCC GTT GTT ATT GAG 1275
 430 His Phe Val Asp Lys Pro Ser Ile Leu TAA ATATTATAGCATTAGAGTACTGTTATATGTTGTTTATTATTAAGCTGTGAATAAA 1364
 CAC TTC GTG GAC AAG CCA AGT ATC TTA

ATAATTATTAAAAAATAAAAAA 1388

FIG. 9B

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 95/14364

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/705 C12N15/81 C12N1/19 C12Q1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOPHYS J, 63 (5). 1992. 1406-1411., MCCORMACK K ET AL 'TANDEM LINKAGE OF SHAKER POTASSIUM CHANNEL SUBUNITS DOES NOT ENSURE THE STOICHIOMETRY OF EXPRESSED CHANNELS' see the whole document	1
X	JOURNAL OF NEUROSCIENCE, 13 (11). 1993. 4669-4679., ZHONG Y ET AL 'Modulation of different K+ currents in Drosophila: A hypothetical role for the eag subunit in multimeric K+ channels' see the whole document	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- '&' document member of the same patent family

Date of the actual completion of the international search

21 March 1996

Date of mailing of the international search report

27 MARCH 1996

Name and mailing address of the ISA

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Fax (+ 31-70) 340-3016

Authorized officer

Gurdjian, D

INTERNATIONAL SEARCH REPORT

Internation Application No
PCT/US 95/14364

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 345, 1990 pages 530-4, E.Y.ISACOFF ET AL. 'Evidence for the formation of heteromultimeric potassium channels in Xenopus oocytes'	1
Y	see the whole document ---	20-26
X	NATURE, vol. 368, March 1994 pages 32-38, R. WILSON ET AL. '2.2 mb of contiguous nucleotide sequence from chromosome III of c.elegans'	1
Y	see abstract; table 2 ---	20-26
Y	SCIENCE , vol. 256, 1992 pages 663-5, H.SENTENAC ET AL. 'Cloning and expression in yeast of a plant potassium ion transport system' cited in the application see the whole document ---	20-26
Y	EP,A,0 615 976 (AMERICAN CYANAMID CO) 21 September 1994 see the whole document ---	20-26
A	PROC NATL ACAD SCI U S A, 86 (12). 1989. 4372-4376., KAMB A ET AL 'IDENTIFICATION OF GENES FROM PATTERN FORMATION TYROSINE KINASE AND POTASSIUM CHANNEL FAMILIES BY DNA AMPLIFICATION' see the whole document ---	10,12
A	US,A,5 356 775 (HEBERT STEVEN C ET AL) 18 October 1994 see the whole document ---	1,10-13
A	NATURE, vol. 362, 1993 pages 127-133, Y.KUBO ET AL. 'Primary structure and functional expression of a mouse inward rectifier potassium channel' cited in the application see the whole document ---	10-13

-/--

INTERNATIONAL SEARCH REPORT

Inv. No. Application No
PLI/US 95/14364

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>NATURE (LONDON), 376 (6542). 1995. 690-695., KETCHUM K A ET AL 'A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem' see the whole document -----</p>	1-13

INTERNATIONAL SEARCH REPORT

Intern. application No.

PCT/US 95/ 14364

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 23-26
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 23-26 refer, at least partially as far it concerns a medical method, to a method of treatment of the human or animal body, the search has been carried out and has been based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inventor's application No

PCT/US 95/14364

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0615976	21-09-94	CA-A- 2112445 JP-A- 6253849	01-07-94 13-09-94
US-A-5356775	18-10-94	NONE	

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